CMFRI Marine Fisheries Policy Series No.10
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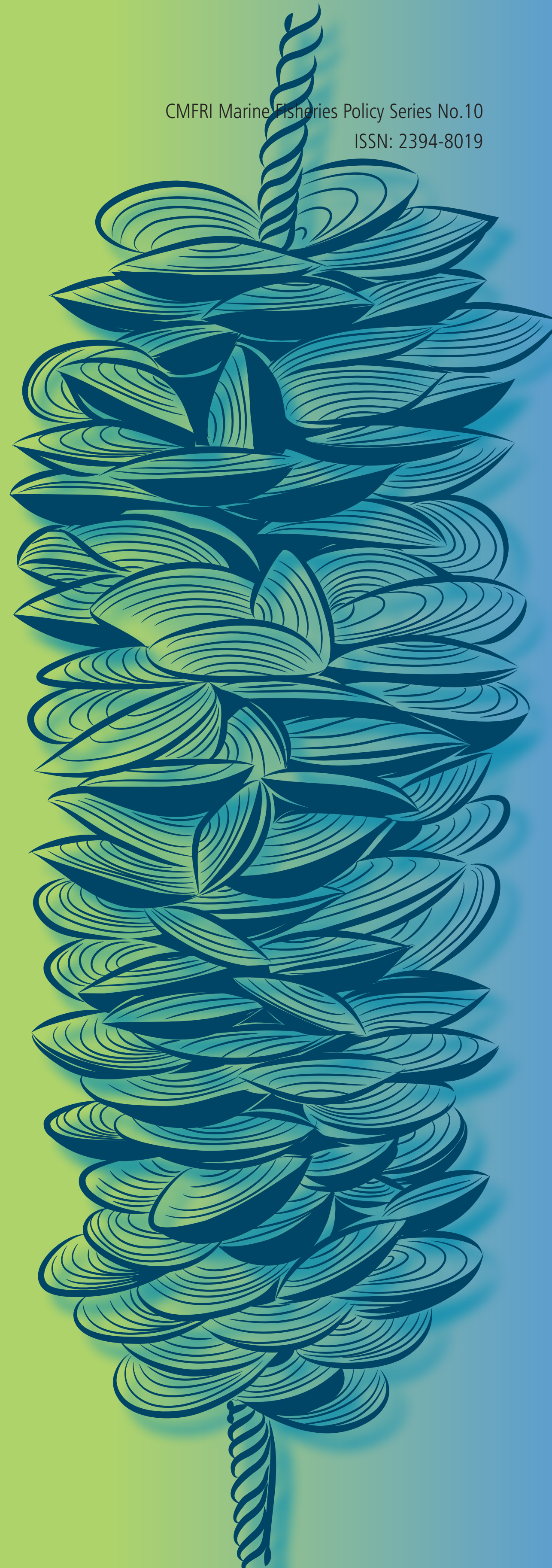
Guidance for **Good Mussel Farming** practices in India based on a case-study from Kerala



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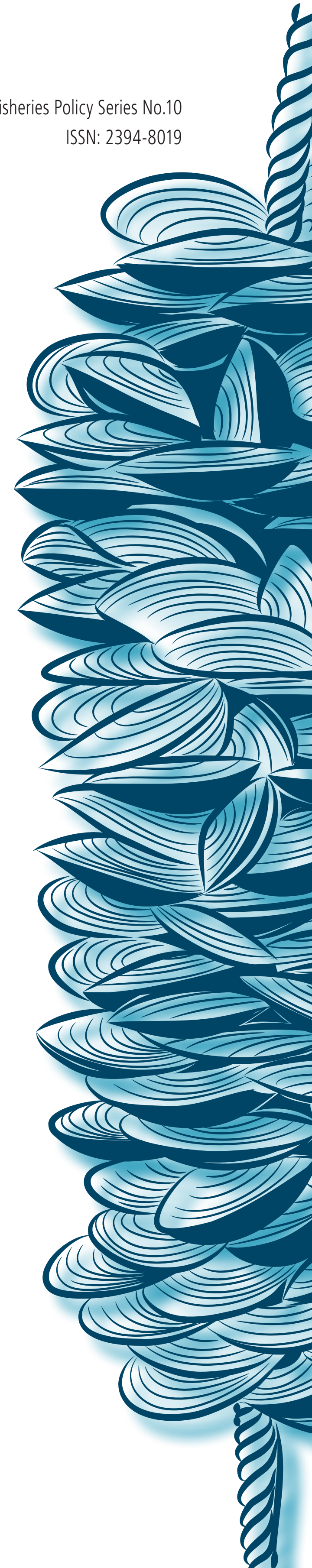

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Dr. A. Gopalakrishnan
Director, ICAR - Central Marine Fisheries Research Institute
Post Box No. 1603, Ernakulam North P.O.
Kochi – 682 018, Kerala, India

www.cmfri.org.in
Email: director@cmfri.org.in
Tel. No.: +91-0484-2394867
Fax No.: +91-0484-2394909

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Executive summary

In India, the adoption of mussel (*Perna viridis*) farming, practice began in 1996 when it was field tested in the shallow backwaters of Padanna in Kasaragod District of Kerala. This paved the way for commercial mussel production from northern districts of Kerala, particularly in Padanna Backwaters by coastal villagers. There was a steady rise in mussel production from estuarine farms till 2008, which was followed by a reduction since 2009. During 2015, this rate of reduction in farmed mussel production was drastic, recording as high as 93.8%, when compared to the 2014 figures. This reduction was not restricted to Padanna Backwaters alone, as there was mortality and decline in production in all northern districts of Kerala in 2015.

Mussel farming sector of Padanna Backwaters is confronted with several problems. With rapid growth in farming the availability of mussel seeds became limiting and farmers sourced seeds from distant locations resulting in poor seed quality at the time of seeding. The supply-demand gap pushed the farmers to compromise on the seed quality. This resulted in tended mussel stocks which were susceptible to stress. The environmental degradation in the farmed area of Padanna due to excessive number of farms per unit area and reduced flushing of water in certain pockets was further burdened by the extremely high ambient air and water temperatures in 2015-16. The prevailing environmental anomaly resulted in higher than normal salinity and high temperature, which compounded the environmental stress on the farmed mussels. This resulted in a crisis in February 2016, leading to stunted growth, high mortality and prevalence of the protozoan parasite, *Perkinsus olseni*.

The CMFRI's scientific team which has been monitoring the mussel farming activity in the area, setup a task force to identify the issues and suggest solutions to farmers and the Government. The adoption of sustainable aquaculture practices in Padanna Backwaters by improving the quality of seeds, enhancing the flushing rates, modifying the farm layout and reducing the farming density per unit area are among the 21 recommendations proposed by CMFRI task force to tackle these challenges. These recommendations are complementary to the global guidelines for Best Aquaculture Practices for mussels. This requires the collaborative effort from the farmers, local administration and the fisheries department.

Contents

1. Introduction

1.1 Growth of mussel farming sector in the estuarine waters of Malabar area

In India, the technologies for farming the green mussel, *Perna viridis*, was developed during 1970s (see Box 1) and was subsequently tested for feasibility at various locations along the country's southeast and southwest coasts by Central Marine Fisheries Research Institute (CMFRI). However, the technology was not adopted by fishers because of risks associated with sea farming such as poaching, weather-related damage/loss to farm structures, relatively higher investment cost in the sea, logistics and lack of awareness. Adoption of this aquaculture practice by coastal villagers began only in 1996 when it was field tested in shallow backwaters of Padanna in Kasaragod District of Kerala.

Although the technology for mussel farming has been demonstrated in several locations within Kerala State and in different maritime States of India, the diffusion of the technology was predominantly in northern districts of Kerala, particularly in Padanna Backwaters located in Kasaragod District. The successful adoption of mussel farming by coastal fishers in Kerala is the result of a combination of factors, chiefly, the availability of water bodies suitable for estuarine farming of mussels, high rate of literacy and education, the proximity to major mussel markets and high levels of mussel consumption in the area, and a unique synergy between technology developers, promoters and credit advancers (Kripa and Mohamed, 2008).

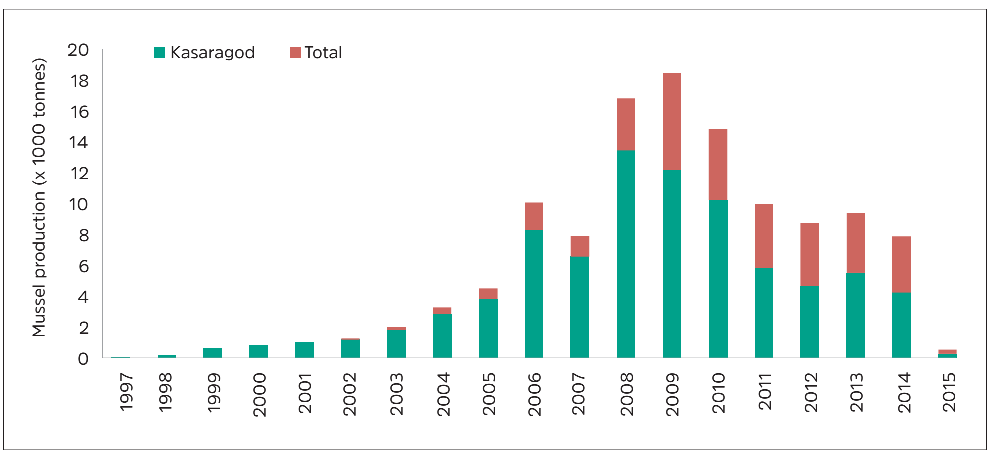


Fig. 1 Farmed mussel production in India

As a result of a concerted approach, coupled with novel extension techniques, commercial bivalve farming became established in the States of India, Kerala and subsequently in Karnataka. During this process, the entire gamut of bivalve farming operations such as site identification, seed and spatfall calendars, remote setting, mechanization in seeding and harvesting, quality and depuration protocols, ready-to-eat and ready-to-cook products, organic farming protocols and environmental impact assessments were worked out. This development scenario has been highlighted as a role model for other states and developing nations where a similar hydrological, social, and market environment exists (Mohamed *et al*., 2015).

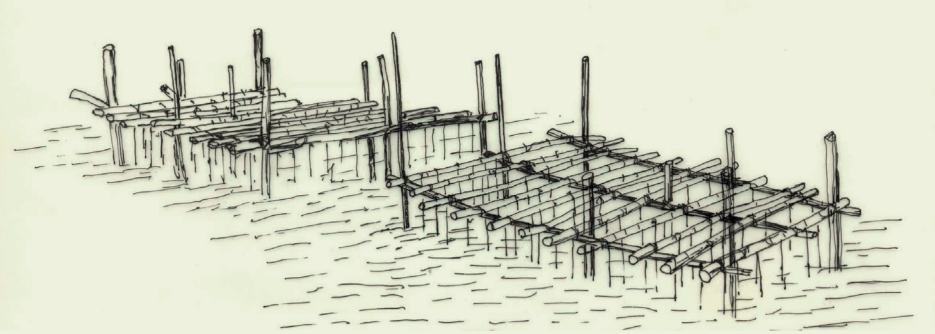
Due to this wide-spread adoption of the farming technology, especially by women self-help groups, (Kripa and Surendranathan, 2008), the mussel production in Kasaragod increased from 2 tonnes in 1996 and peaked to 13,431 tonnes by 2008 (Fig.1). The farmed mussel production in the region witnessed a steady decline from 2009, while the production share increased in other districts of Kerala. However, in 2015, the production in all districts dipped to an all-time low, and the mussel production estimate was 533 tonnes, 93% less than 2014 figures.

This document presents the results of a study undertaken to understand the reasons

Box1: Mussel Farming Methods

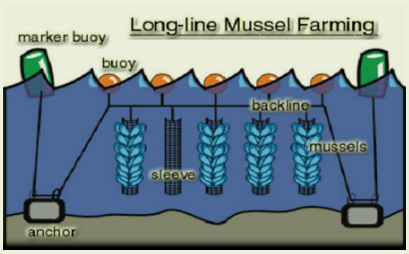
1. Rack Method

This method is especially suited for estuaries and shallow seas. Bamboo or casurina poles are driven into the bottom spaced 1-2 m apart. These stakes are connected horizontally with poles. The horizontal poles should be above the level of water at high tide. Seeded rope can be suspended into the water for farming from these poles. Four seeded ropes can be suspended from one square metre area of the rack.



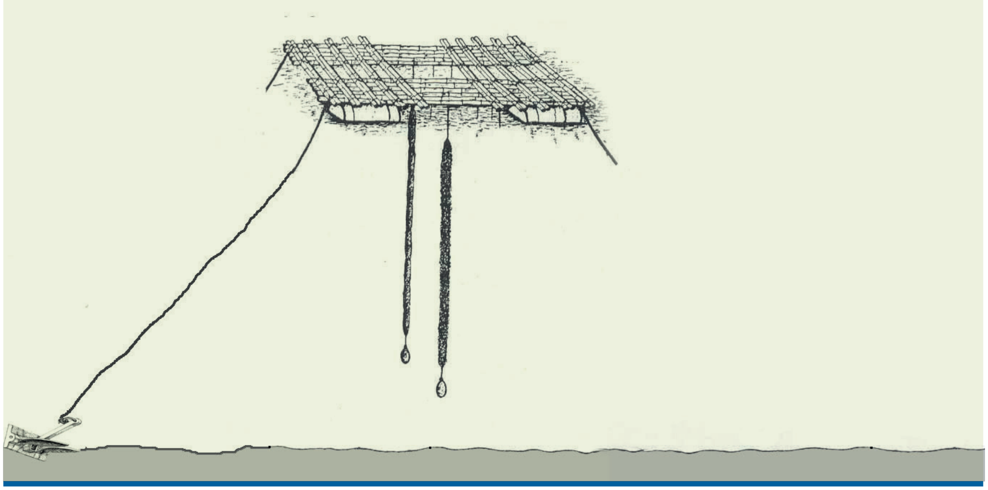
2. Longline Method

This method is considered ideal for unprotected open sea conditions. Synthetic rope of 16-20 mm diameter is used for the longline (main line). The main line is supported with 200 litre empty barrels tied to it, spaced at 5 m. The longline and barrels are anchored in position at either ends using concrete blocks and nylon ropes. Seeded ropes are suspended from the longline.



3. Raft Culture

Ideal for open sea conditions which are not rough. Square or rectangular rafts are made with sturdy bamboo or casurina poles. Buoyancy for the raft is given by tying 5 barrels of 200 litre capacity (metal oil barrel painted with anticorrosive paint or synthetic barrel). Ideal size of the raft is 5x5 m. The rafts are to be positioned at suitable site in the sea using anchors (grapnel, granite, concrete). Four seeded ropes can be suspended from one square metre area of the raft.



for the continuous decline in production of farmed mussels in Padanna Backwaters, and to provide general guidance for good mussel farming practices in estuarine production systems in tune with the carrying capacity of the backwater system.

1.2 Present status of the mussel farming areas in Padanna Backwaters

The number of individual and group farms in Padanna Backwaters was 805 and 192 respectively during 2015-16 (source: State Fisheries Department, Kerala). The number mussel farms under the different Blocks in the area is detailed in Table 1.

Table 1. Details of mussel farms in Padanna during 2015-16 farming season (source: Department of Fisheries, Kerala)

Sl No	Name of the Block	Number of ropes	Production expected (tonnes)	Number of individual farms	Number of society (groups)	Number of farms (individual + Groups)
1	Cheruvattur	8120	81.2	11	25	36
2	Padanna Kadappuram	12693	126.9	41	32	73
3	Padanna	47582	475.8	267	69	336
4	Vadakkekkad	12791	127.9	63	8	71
5	Veliyaparambu	2036	20.3	12	1	13
6	Edayillakadu	50612	506.1	230	29	259
7	Thrikkaripur	3444	34.4	18	6	24
8	Madakka	26390	263.9	78	11	89
9	Kannuveedu	12570	125.7	85	11	96
Total		176238	1762.3	805	192	997

Several problems emerged in the mussel farming sector of Padanna Backwaters leading to a crisis in February 2016. This led to a reduction in the actual production of farmed mussels from Padanna that was estimated at only 264 tonnes during the period.

1.3 Emergence of problems in mussel farming

The farmed mussel production from the area, post 2009 period witnessed a steady decline. The decline in production until 2015 was at a rate of 16-43%, subsequently reaching an all-time low of 264 t in 2016. The reduction in mussel production from 2015 to 2016 was drastic (93.8%). However, the 2016 decline in production was not restricted to Padanna Backwaters alone, as there was mortality and decline in production in all the northern districts of Kerala.

During 2014 period, Edayillakadu area in Padanna Backwaters had high mortality due to reduced flushing of waste in farming area which also lead to eutrophication of the water body. Consequent to this, CMFRI had undertaken a study in 2014, and provided an advisory to the local administration and fisheries department to address this issue.

With rapid growth in farming the availability of mussel seeds became limiting and farmers sourced seeds from distant locations resulting in poor seed quality at the time of seeding. Seeds were sourced from Kollam (~490 km) in Kerala and Malpe (180 km) in Karnataka and transported in bulk quantities during daytime. Likewise, the increase in seed demand coupled with the inability to meet bulk requirement during November to January months, resulted in exorbitant seed prices. The supply-demand gap pushed the farmers to compromise on the seed quality. This resulted in tended mussel stocks which were susceptible to stress.

The problems in the mussel farming sector were related to poor quality seeds, excessive number of farms per unit area, reduced flushing, environmental stress due to high temperature and salinity, stunted growth and high mortality and prevalence of the protozoan parasite, *Perkinsus olseni*.

2. Approach to address the issues related to mussel farming

2.1 Data collection, surveys and sampling areas

For precisely identifying the area (in sq.m.) in which mussel farming is carried out in the Padanna Backwaters, the location map was captured from Google Earth imageries in December 2015 and the mussel farm area was delineated and then rendered into GIS using ArcGIS software. These maps are shown in Fig 5 to 14. Minor errors in the estimated area farmed may occur due to cloud cover and late start of farming in some areas.

For the *in-situ* experiments to determine filtration rates and food demand of mussels, three stations were selected in Padanna Backwaters and the criteria for the site selection were the rate of water flow, depth of the site, access from shore and the proximity to mussel farms. Based on the above criteria, Koyambram (12°13'14.9"N; 75°08' 11.2"E), Ori (12°11'44.2" N; 75°08'14.9" E) and Edayillakadu (12°07'48.1"N; 75°09'53.0" E) were selected (Fig. 2).

2.2 Environmental monitoring

Water quality parameters from the study sites including dissolved nutrients were determined using standard methods (APHA 2009). Water temperature, pH and salinity were recorded from respective probes attached to a multi-parameter instrument (Eutech CyberScan PCD 650). Diurnal observation on the dissolved oxygen levels in the bivalve farming areas on three hourly intervals was carried out at Padanna Backwaters before stocking mussels and once in a month after stocking the seeds in the raft to study the oxygen budget of the mussel farming area during the farming period.

Levels of chlorophyll *a* were measured using spectrophotometer as per the method of Parsons *et al* (1984) and primary productivity were estimated from the dissolved oxygen values of the light and dark incubated bottles (Gaarder and Gran. 1927). Tidal flow rate in the mussel farms was measured from the water flowing through a digital flow meter fastened around a 50 cm diameter metallic hoop kept below water surface for 10 minutes. Tidal flow rate was calculated using the formula,

$$\text{Flow rate V (m}^3\text{/min)} = \frac{\text{F x 58.8 litre}}{\text{10 x 1000}}$$

Where, F= flow meter reading for 10 min. just below the surface.

Fig. 2 Map showing the sampling locations (filled circles) in Padanna Backwaters

2.3 Tidal volume

The Padanna Backwaters system receives tidal water from Arabian Sea through the bar mouth located at Cheruvathur. The surface area in the water body was estimated from GIS maps for each block. The annual average difference between water levels at high and low tide or the tidal amplitude was multiplied by the surface area of each block and summed for estimating the total tidal volume. The tidal amplitude was calculated from tide table predictions of height of high tide and low tide along the coastal areas of North Kerala and Mangalore. The volume of water flowing in and out of the backwaters was considered for modeling the food supply. The food production in the water volume remaining in the basin was excluded from the model, thus, accounting for the food requirement of the estuarine fauna.

2.4 Food content & supply

Water samples were collected from each station for estimation of particulate organic matter (POM) and chlorophyll-*a* (*chl a*) by using a pump. The chl *a* and POM were used as indices for the quantitative analysis of food available in the farming area.

Chl a content of the water was measured by spectrophotometry after vacuum filtration and extraction in acetone. 500 ml of seawater sample were filtered through Whatman GF/C filter paper and kept in 10 ml of 90% acetone. Magnesium carbonate was added during filtration to retard degradation and enhance filtration efficiency. The pigments were extracted with acetone and extract was centrifuged and *chl a* in the supernatant was determined by spectrophotometry.

The total particulate matter (TPM) was determined by gravimetric method Wong and Cheung (2001). A sub-sample of 500 ml of seawater was filtered through 47 mm GF/C pre-combusted (for 2 h at 450°C) and pre-weighed (±0.001 mg) filter paper to estimate TPM. Salt was removed from the filter by rinsing with distilled water and the filter paper was dried at 110°C and weighed. The TPM was estimated as the weight of residue retained, expressed as mg l^{-1}. The filters used for determination of TPM were ashed in a muffle furnace at 450°C and weighed. Particulate Inorganic Matter (PIM) was determined as the difference in weight of filters before and after combustion. The amount of POM suspended in water was estimated by first removing the suspended material from the water by filtration, followed by either a direct measurement of the amount of carbon retained on the filter or by estimating the amount of carbon present from the weight lost upon heating the filter in excess of 450°C. Concentration of Particulate Organic Matter (POM) or the food content was estimated as the difference between TPM and PIM.

Multiplying the food content level per litre of water by the tidal volume in the backwaters gave the estimates of food supply during tidal exchange.

2.5 Filtration rate & food demand in mussels

An *in-situ* filtration chamber was designed to measure filtration rates in the field as shown in Fig. 3 (Carver and Mallet, 1990). The filtration chamber consisted of a reservoir of 20x10 cm dimension and a grazing chamber of 30x40x20 cm size separated by a partition of 20 cm height. The depth of water in the grazing chamber is maintained at *±1 cm* the height of the partition wall of the reservoir. The chamber was partially submerged in the backwaters, such that the internal water level in the grazing chamber was at the same level as that of the external water level. The experimental chamber was kept in position in the waterbody by using a PVC pipe float around the chamber. Water inlet was placed on the reservoir side so that the inflow was initially allowed to fill the reservoir. Direct pumping of water to the grazing chamber was avoided. Once the reservoir was filled up completely the water was allowed to overflow to the grazing chamber, where the green mussels were placed on a false bottom. Water was pumped out of the grazing chamber using a submersible pump. The flow of water from the reservoir to the grazing chamber was regulated by adjusting the flow rate (outflow) of the pump.

The chambers were placed in the commercial mussel farming areas in Padanna Estuary for the *in-situ* experiments. A generator was operated from a canoe to supply power for the submersible pumps. Mussels were collected from the farm grown ropes suspended from backwaters in the location. They were cleaned, measured, placed in net bags and suspended in water 12 h prior to the experiment. Four experimental chambers were used for the experiment. Fifteen acclimatized mussels were transferred from the net bags and placed over the false bottom in each of the grazing chamber. Three of the four chambers were stocked with 15 mussels and set as the experimental units for determining the filtration rate of mussels and the fourth chamber was set as control without mussels.

The flow rates in the chambers were set at 800 ml min^{-1}. Flow rates were recorded hourly and water samples were collected concurrently for the analysis of chlorophyll and POM for 5 hours. Filtration rates (l h^{-1}) were estimated from the following inputs:

1) Flow rate (l h^{-1}) of water through the experimental chamber (fr)

2) Food availability in the backwaters ($POM_{control}$)

3) Food filtered by the mussel in an hour ($POM_{control}$-$POM_{experiment}$)

Filtration rate = fr x ($POM_{control}$-$POM_{experiment}$/$POM_{control}$)

Multiplying the filtration rate (l h^{-1}) by the average concentration of POM in the backwaters gave estimates of food ration of mussel in an hour.

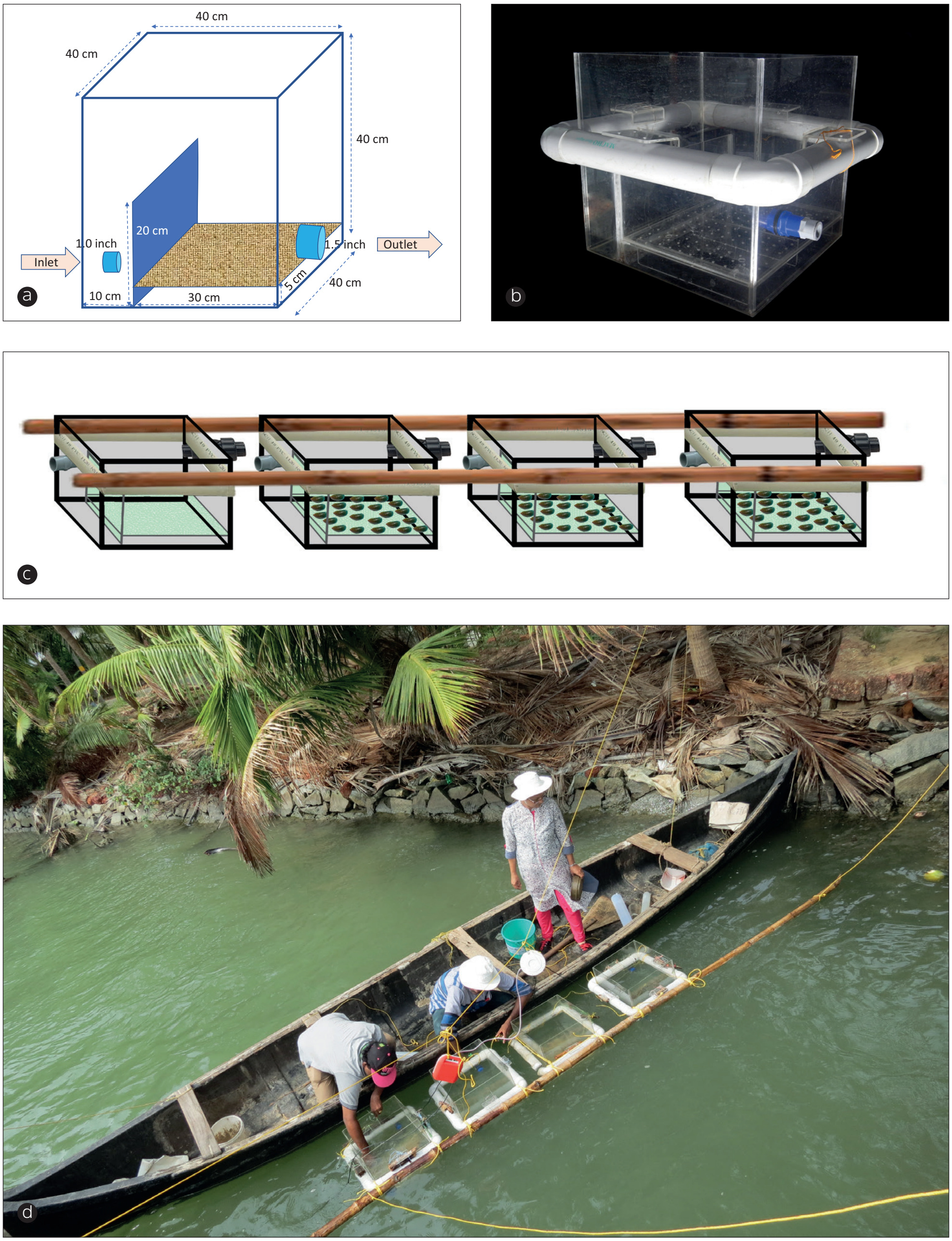


Fig. 3 Filtration chamber a) design b) chamber photograph c) experimental layout with a control chamber without mussel and d) photograph of the experimental layout

2.6 Pathological investigation protocols

Regular samplings were made from the farms during the culture period in 2015 and 2016 for pathological investigation. In addition, dead and moribund mussels were also collected as and when mortalities were reported from the area. Samples were collected and processed as per standard procedures recommended by Office International des Epizooties (OIE) (OIE, 2016). Briefly, tissues of gills, mantle, muscles and digestive tubules were collected. One part was fixed in Davidson's fixative for histologic studies and another was fixed in 90% ethanol for molecular analysis. The remaining part was used for RFTM (Ray's Fluid Thioglycolate Medium) culture assay specific for *Perkinsus* sp. and the intensity of infection assessed under Mackin's scale. Positive histology slides were further confirmed by fluorescent *in-situ* hybridization. Samples found positive in molecular analysis were further corroborated by PCR product sequencing and higher bioinformatics analysis.

2.7 Seed sourcing

Though CMFRI has developed successful hatchery production technology of mussel seed (Appukuttan *et al*., 1987), farming is still dependent on the availability and utilization of wild seeds for both intertidal and sub-tidal mussel beds. Unregulated and unsustainable harvesting of wild seed from mussel beds risks future depletion of stocks and a consequent decline of the broodstock needed to ensure further seed production. The mussel beds may also provide a food source for fishes and other aquatic organisms, either directly or indirectly. Improved harvesting techniques of wild seed from intertidal and subtidal mussel beds with appropriate harvesting tools should therefore be carried out. Handling, storage and transportation should aim at higher quality and survival of seeds.

2.8 Seed Quality

The availability of good quality wild seed in sufficient quantity is the main constraining factor in mussel farming. The rapid expansion of mussel farming and localized concentration of mussel farms necessitated identification of new spat collection sites from distant locations. This resulted in unorganized mussel seed trade involving middlemen/agents. The supply-demand gap pushed the farmers to compromise on the seed quality. Standard protocols for spat collection and storage during transportation are not in place resulting in unreliable seed quality.

CMFRI focused on developing a mussel seed quality test for the mussel farmers for quality assurance. The ability of seed mussels to attach by byssus threads to the culture rope was tested for developing a commercially useful method for categorizing the mussel seed quality

3. Results

3.1 Farming area estimation

The total backwater area available in Padanna for mussel aquaculture was estimated at 9,204,990 sq.m. GIS results showed that the farmed area was 190,262 sq.m., which is 2.06% of the total area. The farmed area was distributed under 9 Blocks, Cheruvattur, Padanna Kadappuram, Padanna, Vadakkekkad, Veliyaparambu, Edayillakadu, Thrikkaripur, Madakka, Kannuveedu in four panchayats.

Table 2. Estimated farming area in Padanna Backwaters

Sl No	Name of the Block	Area (sq.m.)	Farmed Area in Block (sq.m.)	Farmed Area in Block (ha)
1	Cheruvattur	323989	1973	0.20
2	Padanna Kadappuram	1079351		
3	Padanna	962231	49693	4.97
4	Vadakkekkad	1887899	21951	2.20
5	Veliyaparambu	446204	3923	0.39
6	Edayillakadu	1572939	57588	5.76
7	Thrikkaripur	734146	8576	0.86
8	Madakka	1266798	27096	2.71
9	Kannuveedu	931433	19462	1.95
Total		9,204,990	190,262	19.02

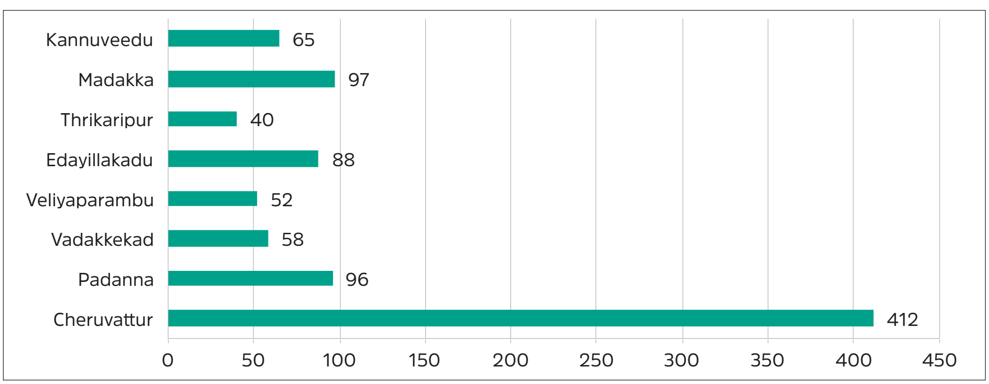


Fig. 4 Block-wise number of farming units ha^{-1} in Padanna Backwaters during 2015

The number of farming units ranged from 40 to 412 per ha with high concentrations in Cheruvathur, Padanna, Madakka and Edayillakadu. The total number of farms in the main farming areas such as Edayillakadu, Padanna (Ori) and Madakka was very high and concentrated within a small area. In the listed areas, the distance between the units was only 5 m, whereas, in other locations the distance was 10-25 m (Table 3).

Table 3. The distance between the farming units in Padanna Backwaters

	Area	Distance from shore (m)	Distance between farms (m)
1	Cheruvattur	5-10	8.1
2	Padanna	20-500	4.7
3	Thekkekkad and Vadakkekkad	20-500	4.8
4	Veliyaparambu	10-20	4.2
5	Edayillakadu	25-50	7.5
6	Thrikkarippur	10-20	11.5
7	Madakka	20-30	3.7
8	Kannuveedu	15-20	6.2
	Average		6.3

The maps of mussel farming area under different blocks in Padanna Backwaters are given below:

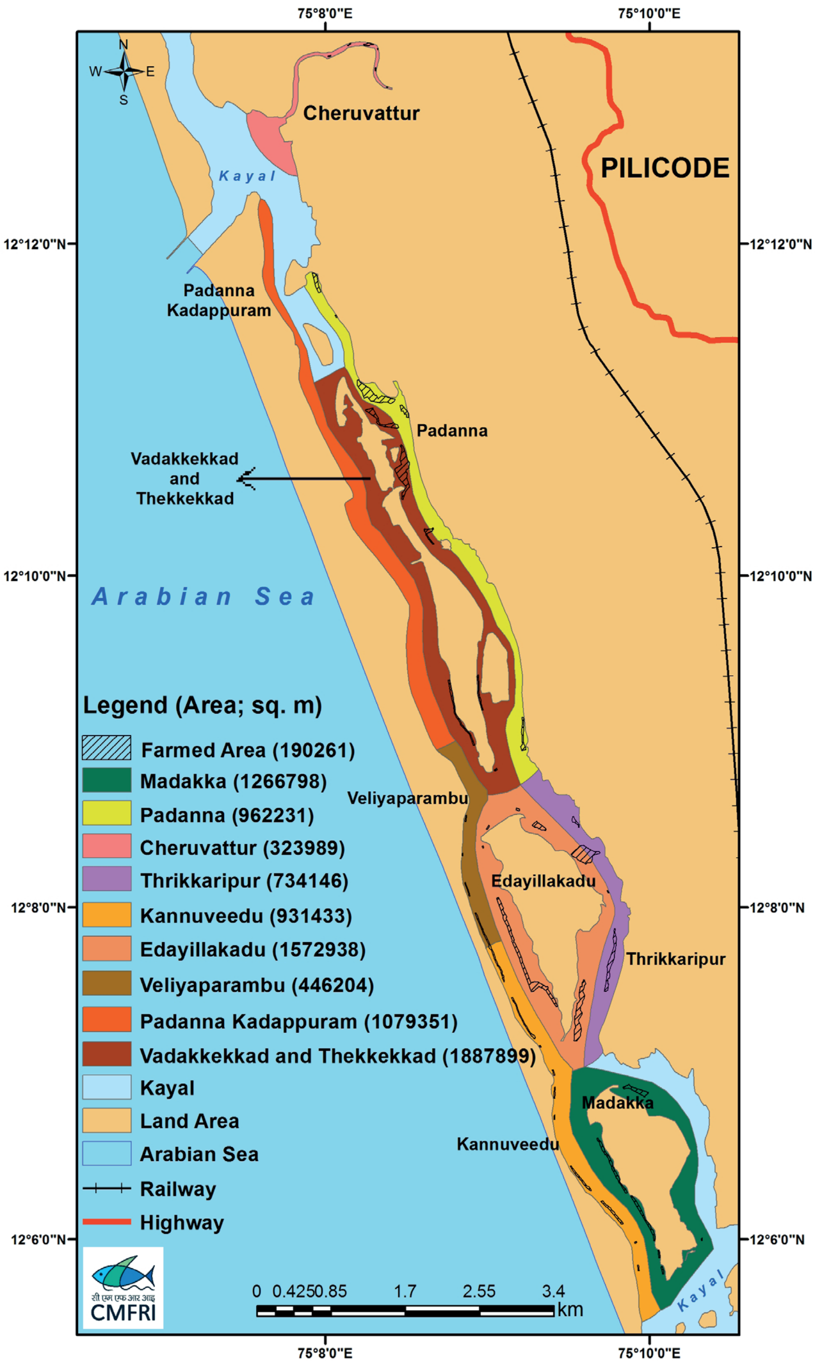


Fig. 5 Composite map of mussel farming areas in Padanna Backwaters

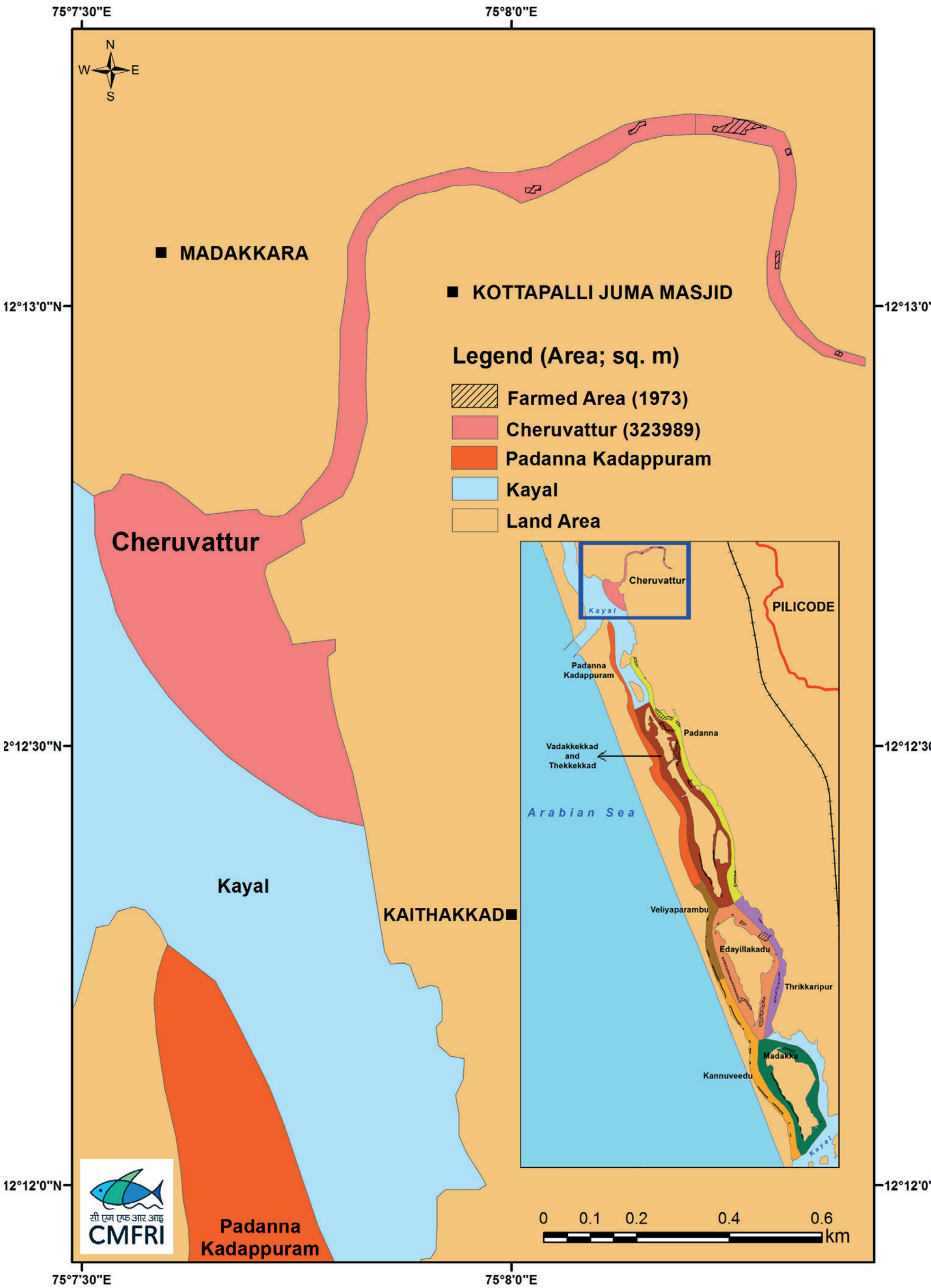


Fig. 6 Map showing mussel farming areas in Cheruvattur

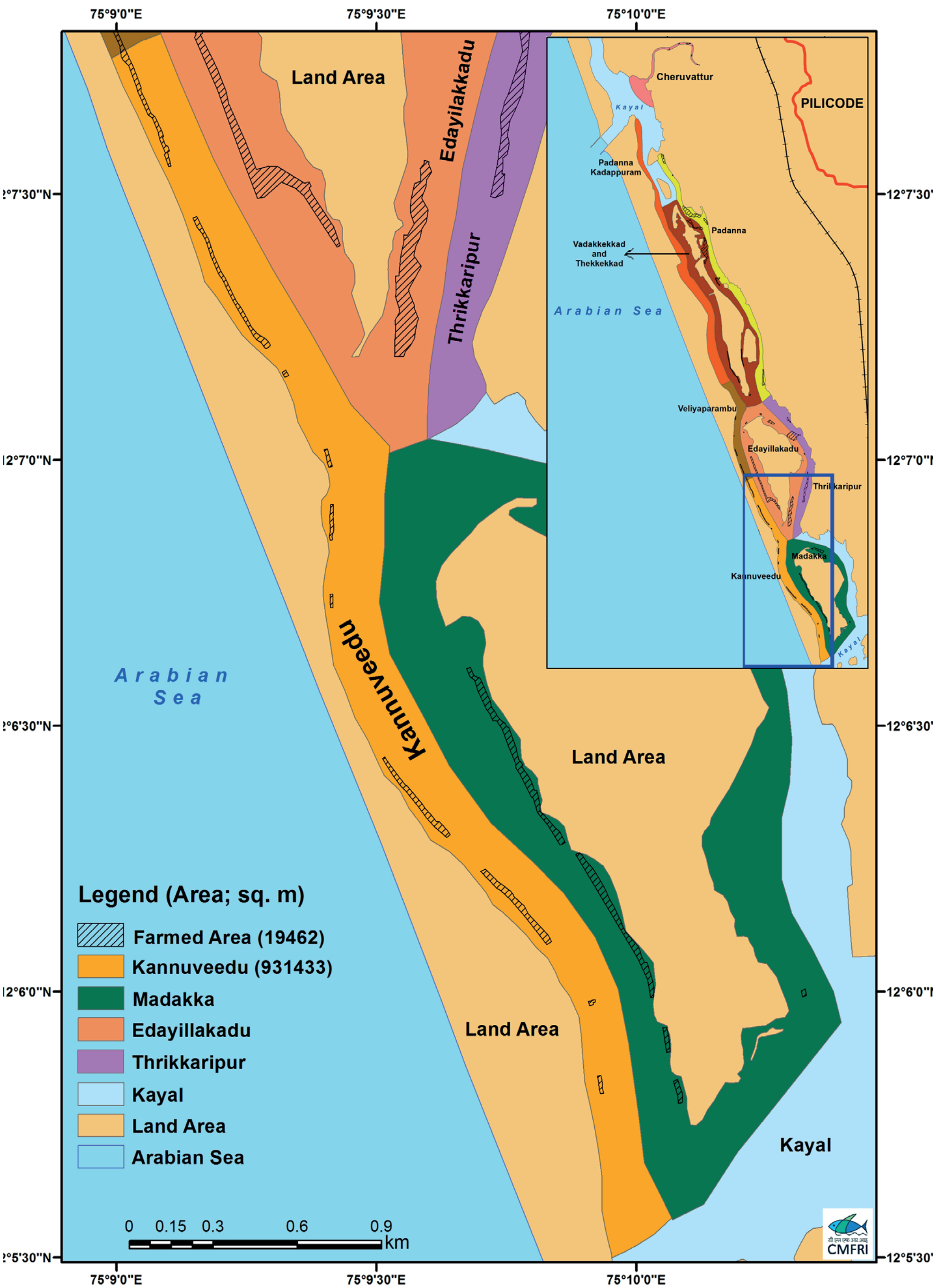


Fig. 7 Map showing mussel farming areas in Kannuveedu

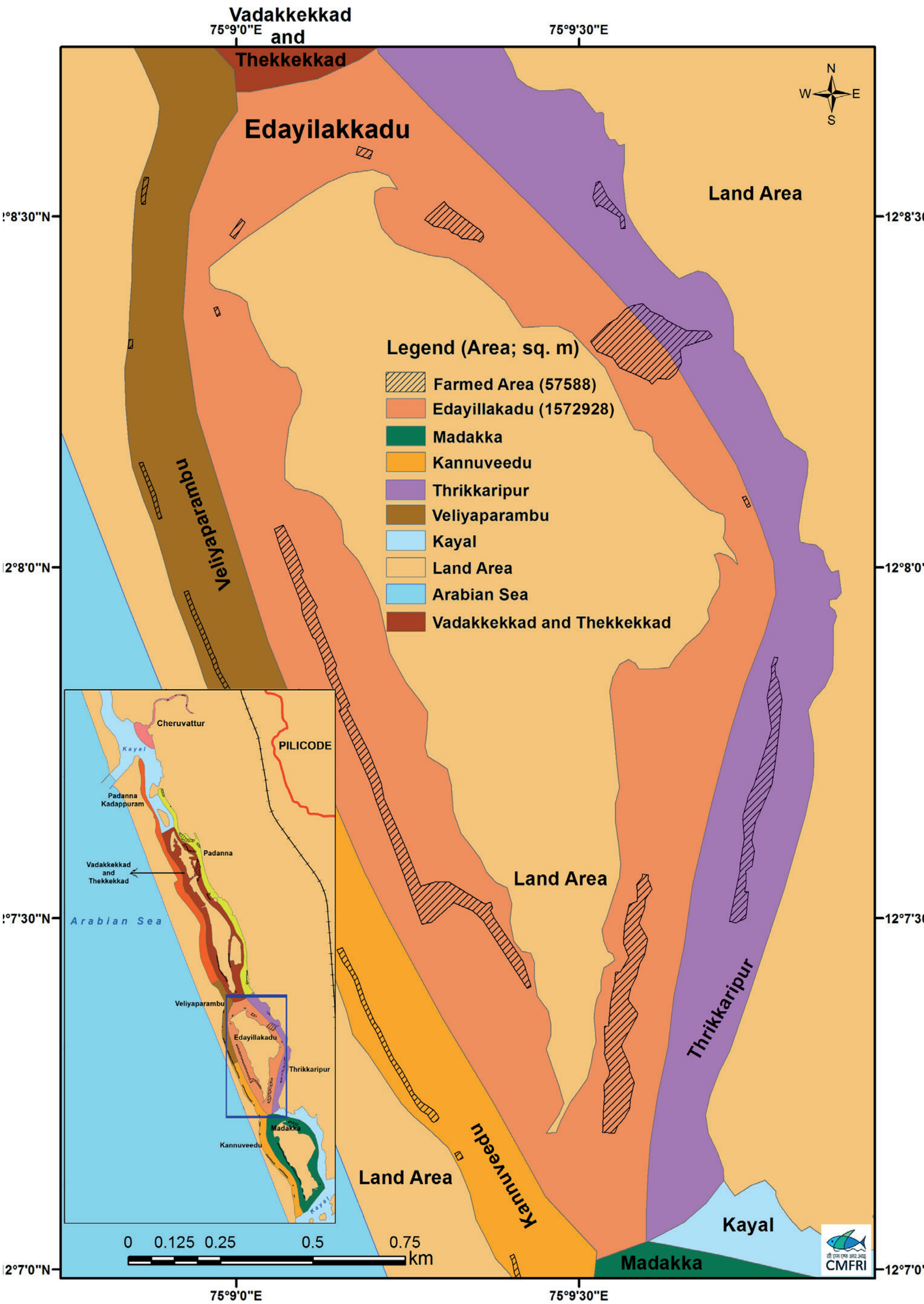


Fig. 8 Map showing mussel farming areas in Edayillakadu

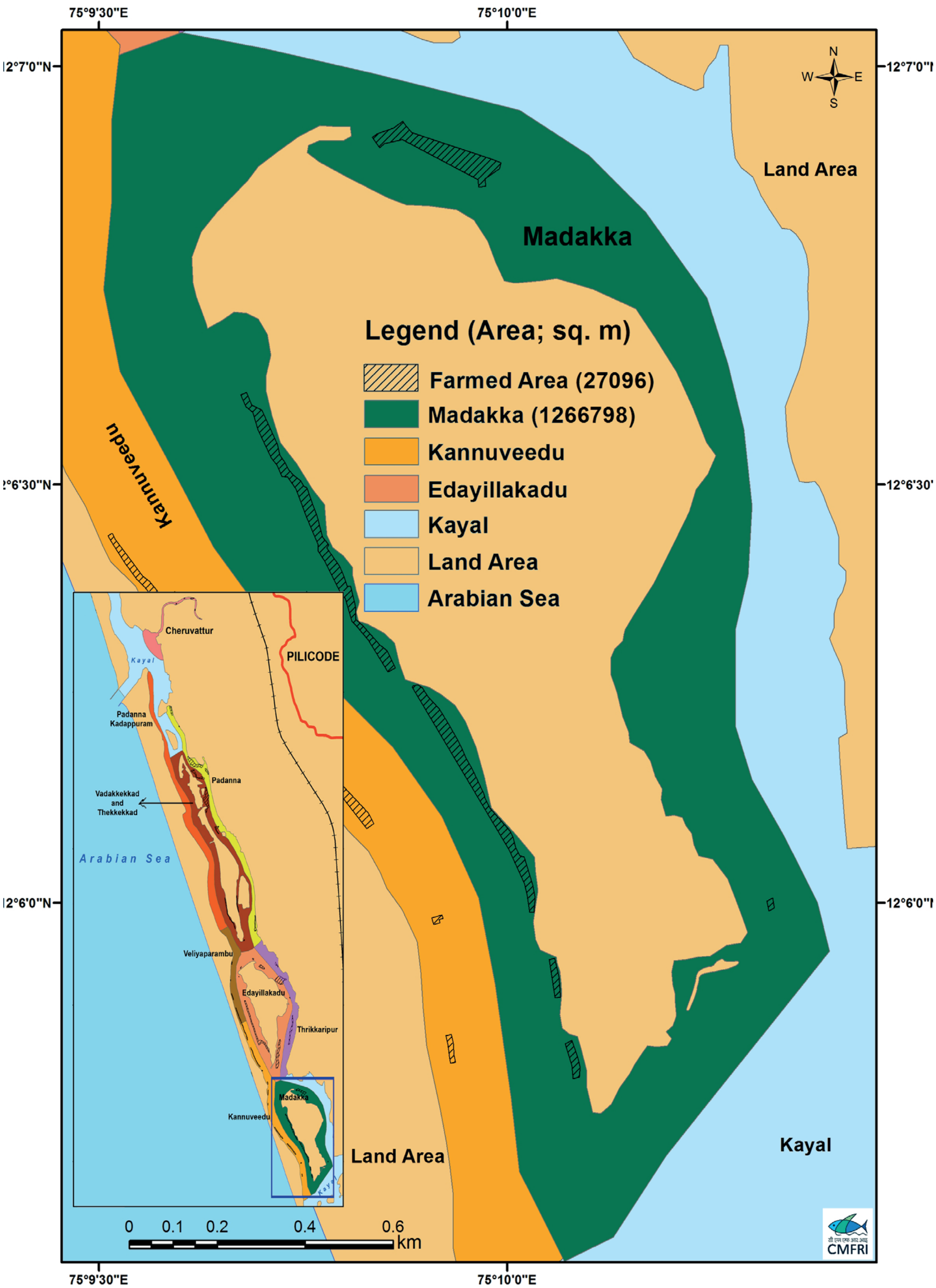


Fig. 9 Map showing mussel farming areas in Madakka

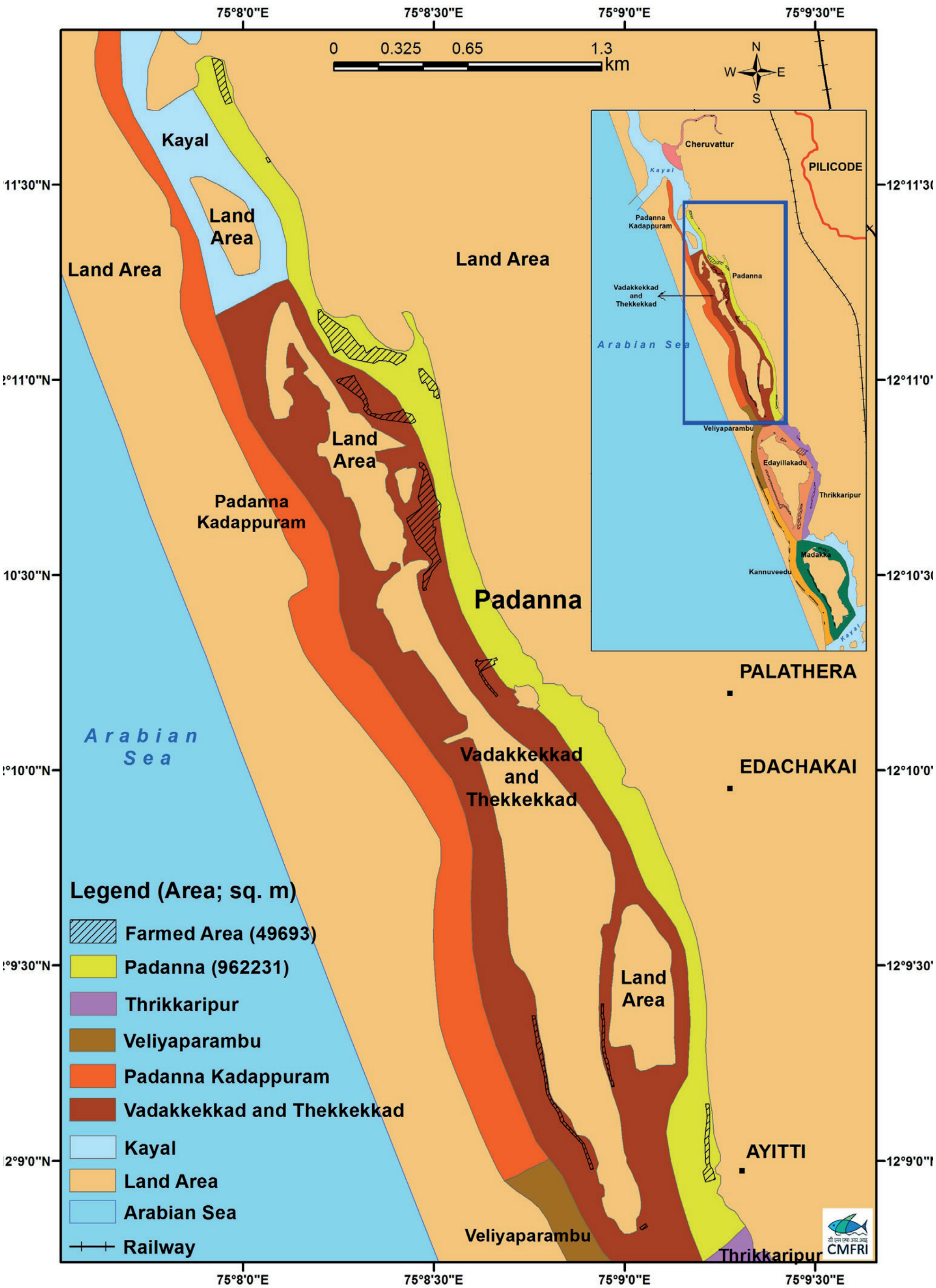


Fig. 10 Map showing mussel farming areas in Padanna

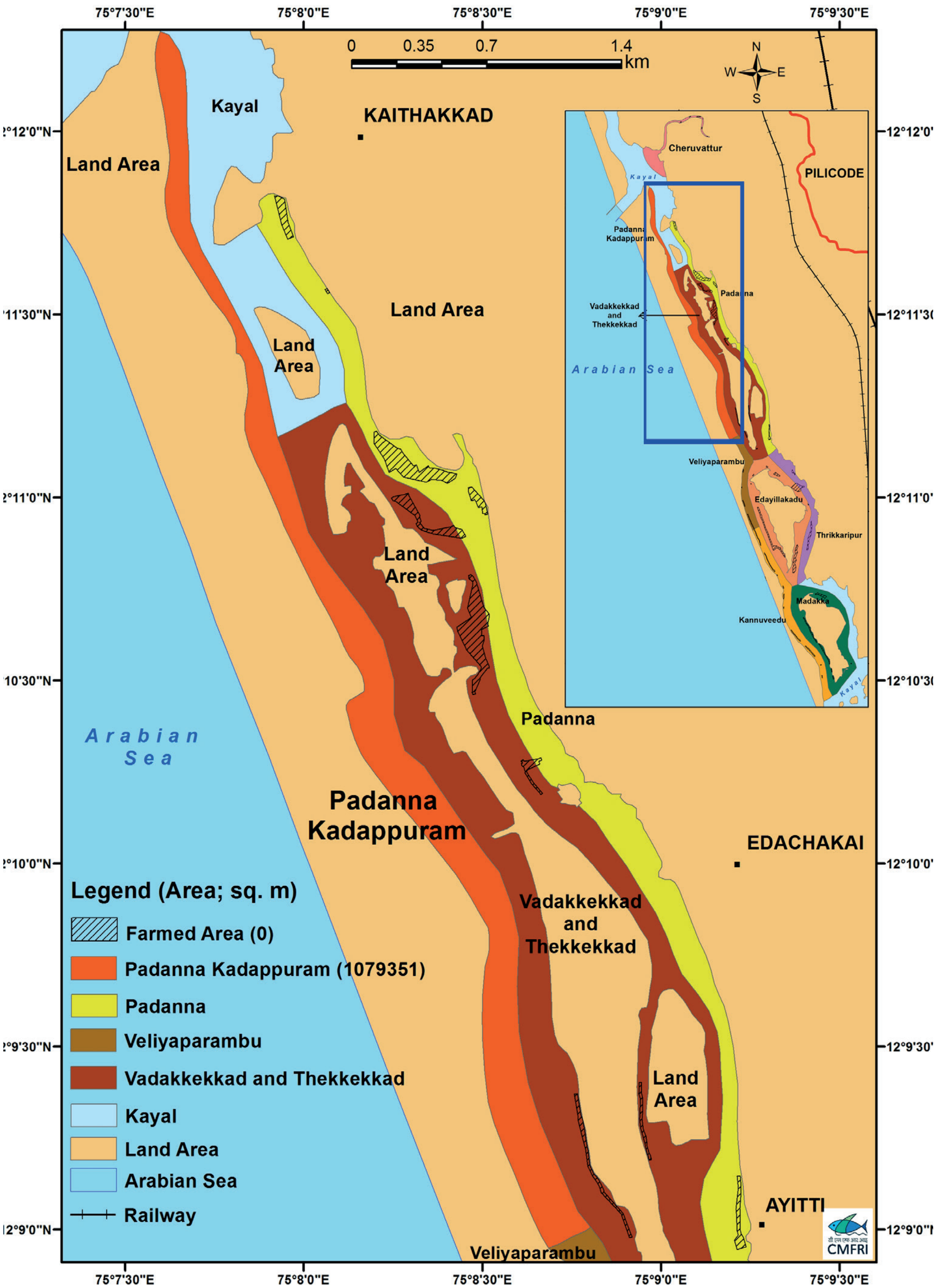


Fig. 11 Map showing mussel farming areas in Padanna Kadappuram

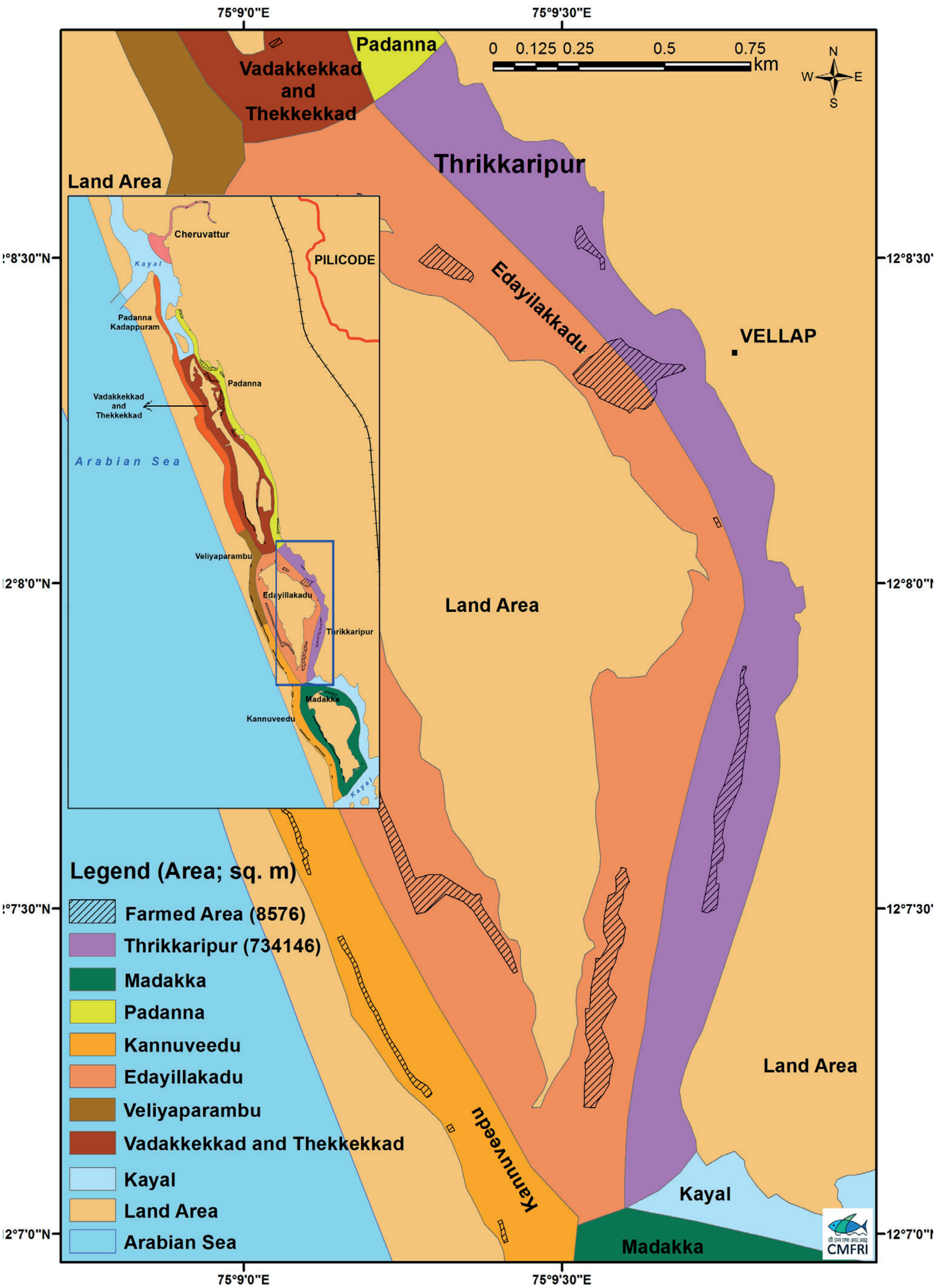


Fig. 12 Map showing mussel farming areas in Thrikkaripur

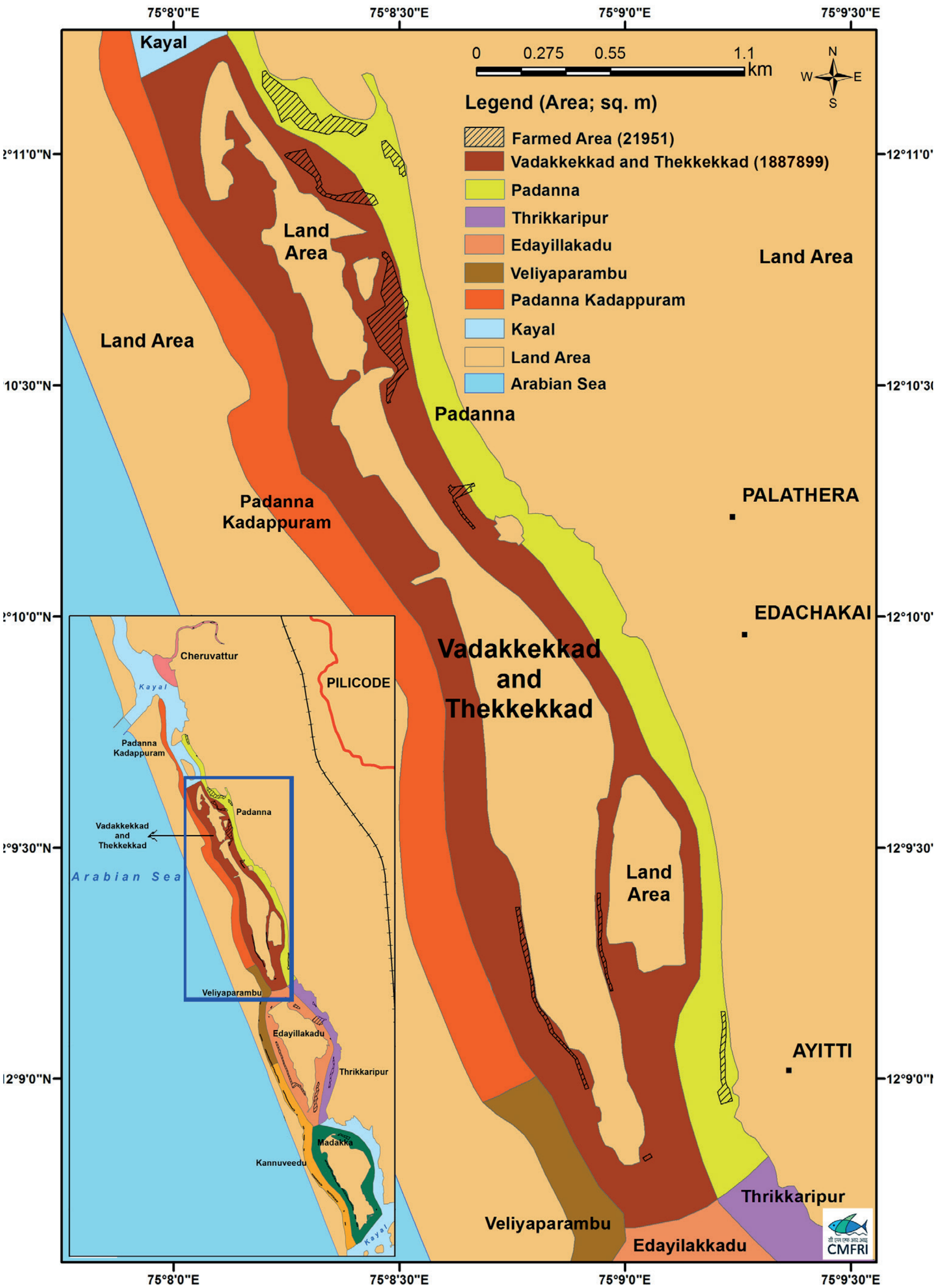


Fig. 13 Map showing mussel farming areas in Vadakkekkad and Thekkekkad

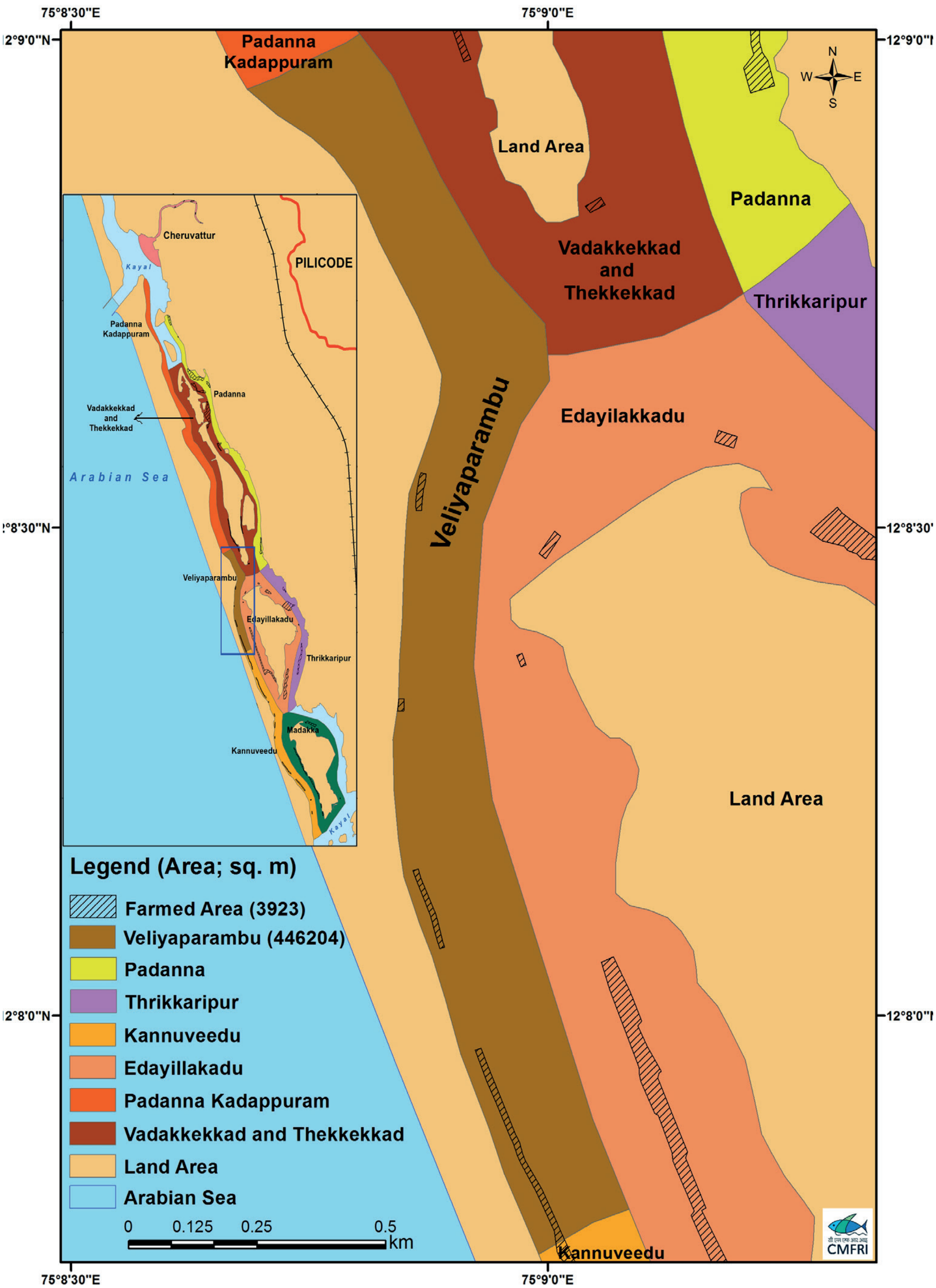


Fig. 14 Map showing mussel farming areas in Veliyaparambu

3.2 Environmental monitoring

The general hydrography of the three sites selected for *in-situ* experiments, Ori, Koyambram and Edayillakadu, were distinct.

The site I (Ori) registered a pH of 7.39 and fairly good oxygen values but low primary productivity. While the site 2 (Koyambram) recorded maximum water temperature, salinity lower than other two sites and maximum primary productivity. However, the Site 3 (Edayillakadu) was distinct from other sites with maximum salinity, lowest pH and dissolved oxygen and low primary productivity.

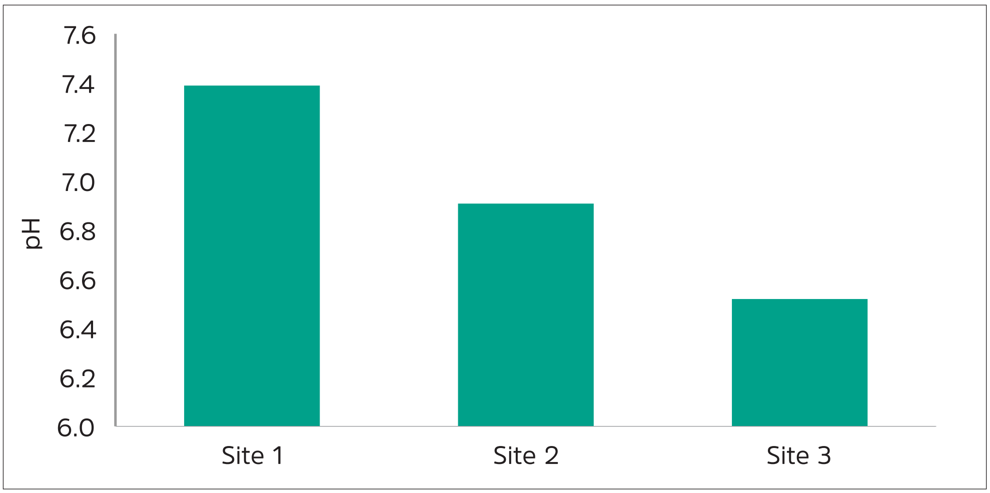


Fig. 15 pH levels in Ori (Site 1), Koyambram (Site 2) and Edayillakadu (Site 3)

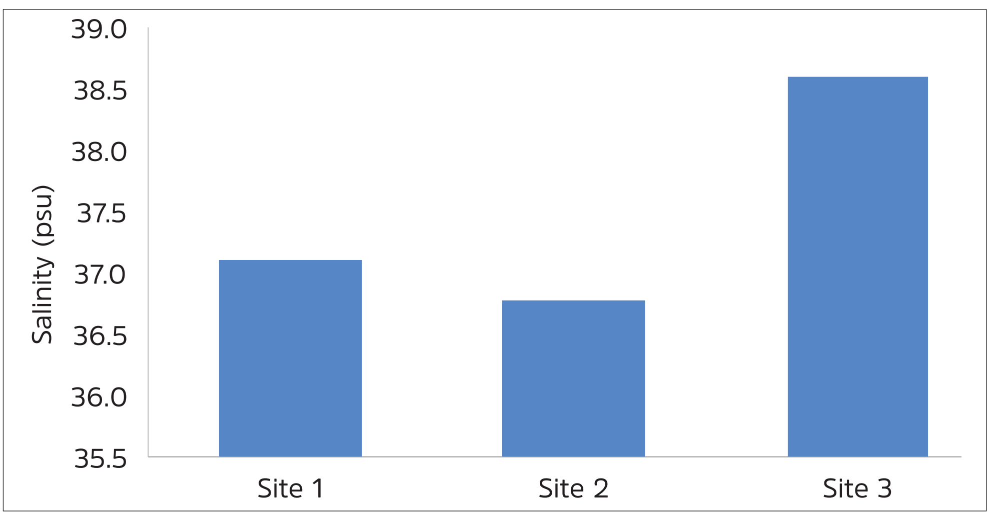


Fig. 16 Salinity levels at Ori (Site 1), Koyambram (Site 2) and Edayillakadu (Site 3)

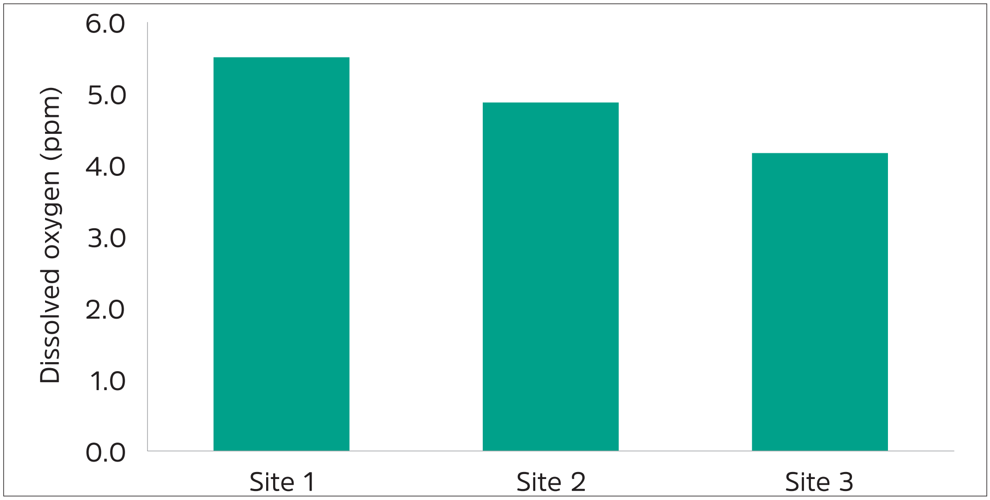


Fig. 17 Dissolved oxygen levels at Ori (Site 1), Koyambram (Site 2) and Edayillakadu (Site 3)

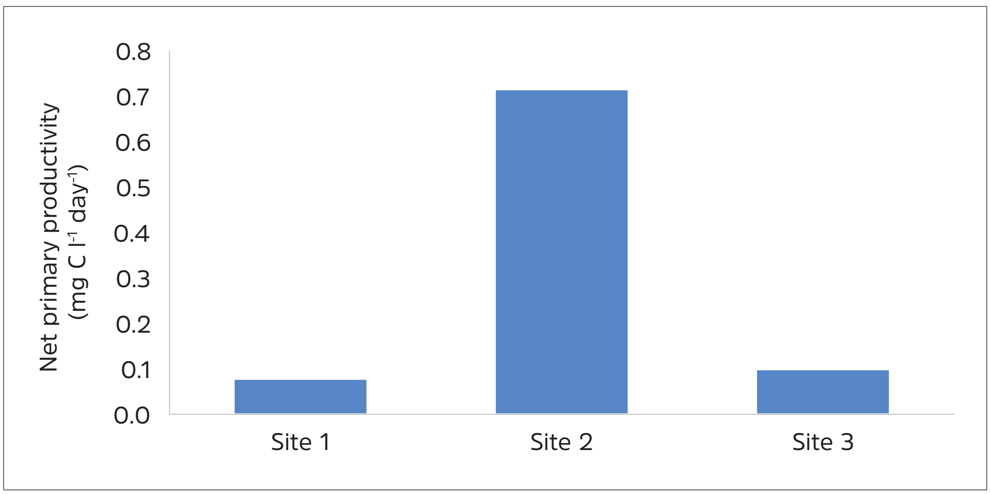


Fig. 18 Net Primary Productivity at Ori (Site 1), Koyambram (Site 2) and Edayillakadu (Site 3)

Flow rate: The flow rate of water at the Ori was 15.3 l min^{-1} during high tide and at 17.64 l min^{-1} during the low tide. At Koyambram the flow rate was 3.53 l min^{-1} and at Edayillakadu was 2.55 l min^{-1}.

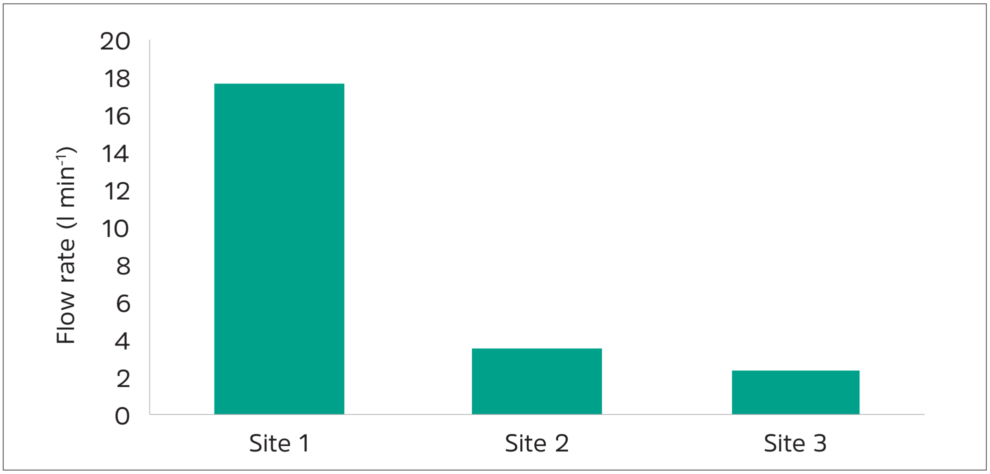


Fig. 19 Flow rate at Ori (Site 1), Koyambram (Site 2) and Edayillakadu (Site 3)

3.3 Tidal volume

The depth of Padanna Backwaters ranged from 1.5 to 3 m during low tide in different Blocks. The tidal amplitude ranged from 0.81 m to 0.96 m. The incoming tidal water volume in Padanna Backwaters ranged from 15.6 x 10^6 to 17.7 x 10^6 m^3 day^{-1} under two diurnal cycles.

3.4 Suspended matter in the farming area

Chlorophyll-a (*Chl a*) concentrations ranged from 7.5 mg m^{-3} in Edayillakadu to 13.34 mg m^{-3} in Koyambram. Total particulate matter (TPM) and particulate inorganic matter was highest in Edayillakadu. However, organic content in the suspended

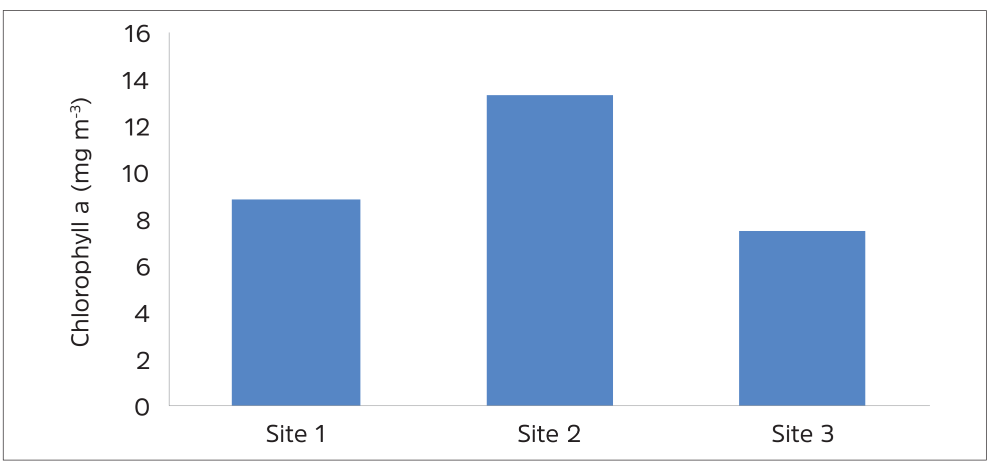


Fig. 20 Chlorophyll-a level at Ori (Site 1), Koyambram (Site 2) and Edayillakadu (Site 3)

matter was higher in Koyambram due to the higher primary productivity (*Chl a*). The TPM level in the backwaters ranged from 15 to 22.4 mg/l.

The chlorophyll-a content and POM levels indicates that the food availability in Padanna Backwaters was adequate for mussel farming, though localized over grazing cannot be discounted near racks in areas with reduced water movement.

Fig. 21 Particulate Organic Matter at Ori (Site 1), Koyambram (Site 2) and Edayillakadu (Site 3)

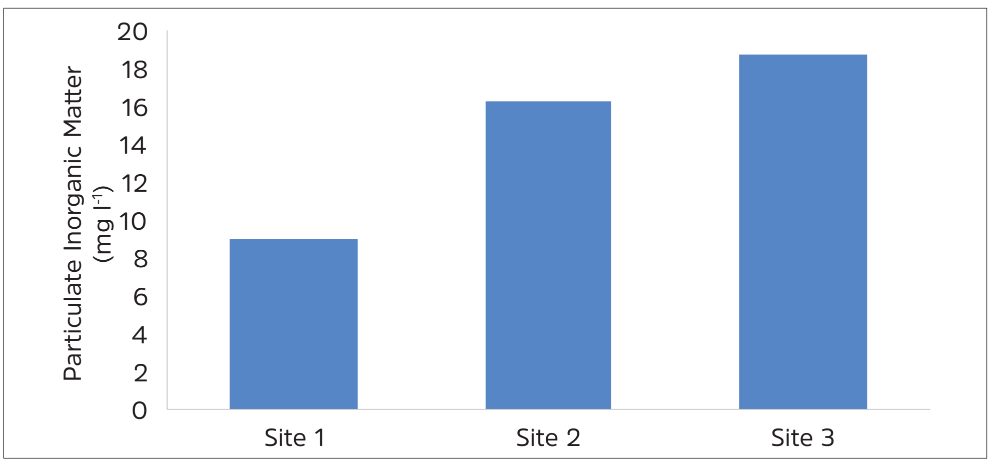


Fig. 22 Particulate Inorganic Matter at Ori (Site 1), Koyambram (Site 2) and Edayillakadu (Site 3)

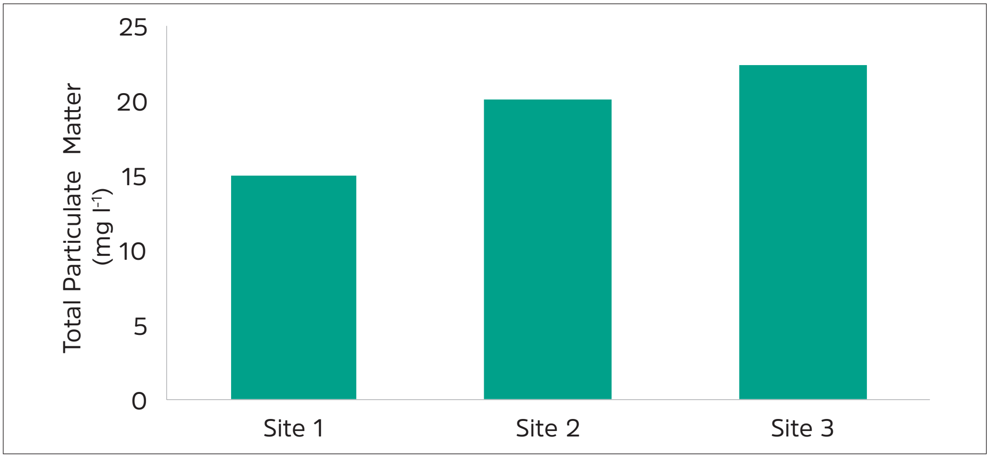


Fig. 23 Total Particulate Matter at Ori (Site 1), Koyambram (Site 2) and Edayillakadu (Site 3)

3.5 Filtration rate

Mussels of 65 to 68 mm length were used in the experiment, corresponding to 1.0-1.4 g of dry tissue weights. The difference in dry weight of mussels among the sites corresponded with the condition index.

During the 4-5h *in situ* experiment the average flow rate of water through the chamber was 788±117 ml min^{-1}. Filtration rate ranged from 1.4 to 2.1 $l.h^{-1}$ (Table 11). The *in situ* filtration rates of mussels were 6-fold lower than experimentally derived peak filtration rates under laboratory conditions.

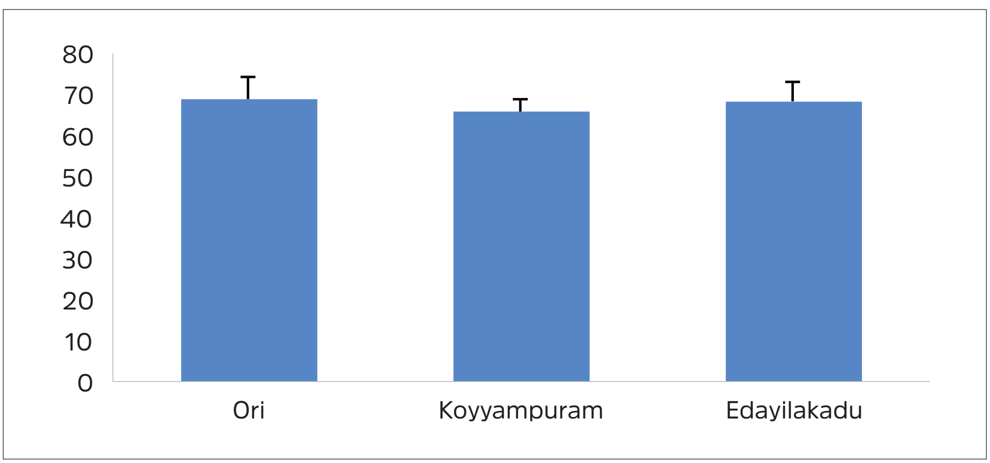


Fig. 24 Mean length of green mussel used for the experiment

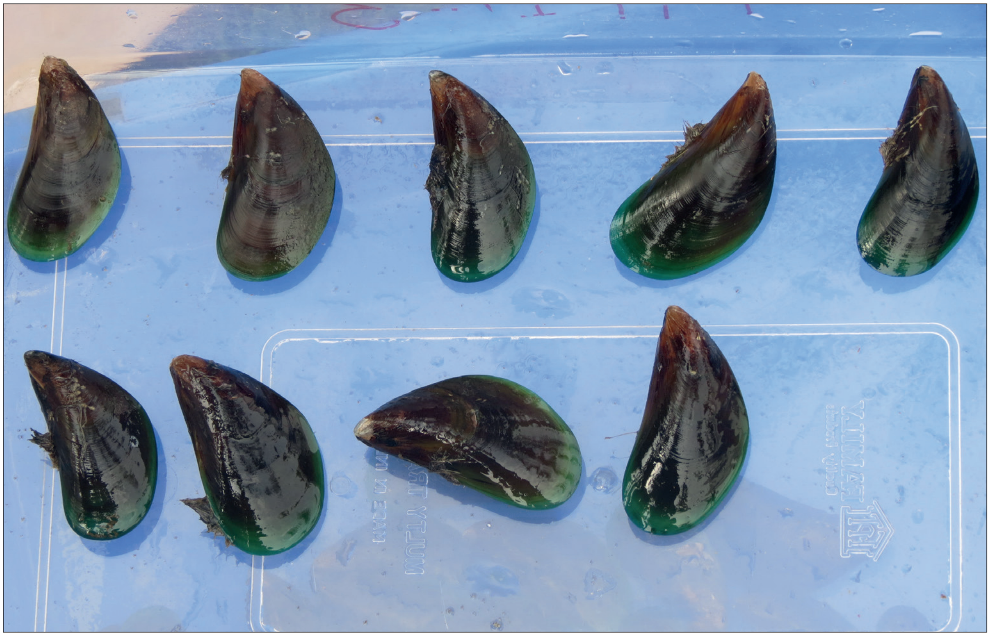


Fig. 25 Green mussel used for the experiment

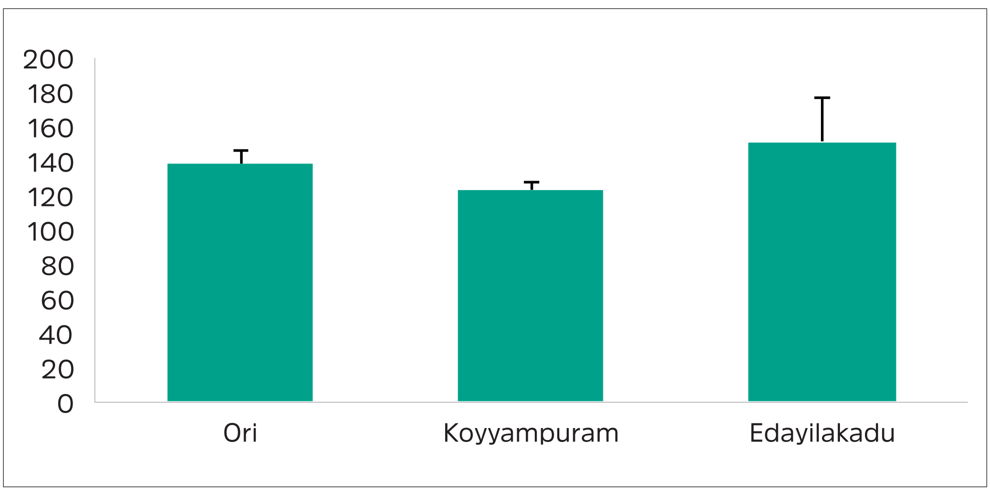


Fig. 26 Condition Index of mussel

Table 4. Particulate organic matter (POM), food supply, filtration rate and estimated food demand of mussels in Padanna Backwaters

Parameters	Cheruvattur	Padanna Kadappuram	Padanna	Vadakkekkad and Thekkekkad	Veliyaparambu	Edayilakkadu	Thrikkaripur	Madakka	Kannuveedu	Total
Area of the Block (sq. m)	323989	1079351	962231	1887899	446204	1572939	734146	1266798	931433	9204990
Farmed Area in Block (sq. m)	1973	0	49693	21951	3923	57588	8576	27096	19462	190262
POM levels (mg l-1) (Food content)	5.2-7.2									
Food supply (kg POM day-1)	96,855-109,576									
Filtration rate (l h-1)	1.4-2.1									
Food demand/ kg mussel (g POM day-1)	8.3-12.5									

From the estimates of food supply and food demand in the above table it is clear that considerable mussel biomass can be supported in the ecosystem. But these are preliminary estimates and more detailed models which account for other POM consumers in the ecosystem need to be developed to reach firm conclusions.

3.6 Pathological investigations

Fig.27 Gaping mussel

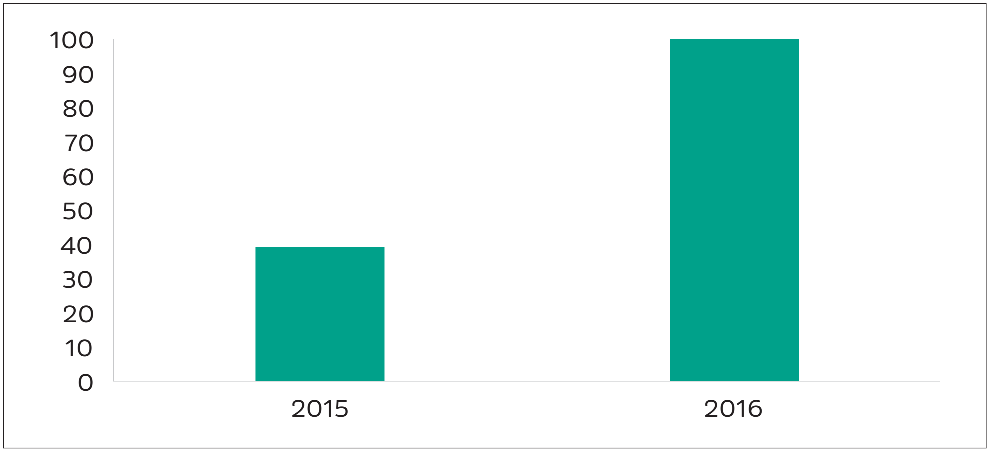


Fig.28 Prevalence of *Perkinsus olseni*

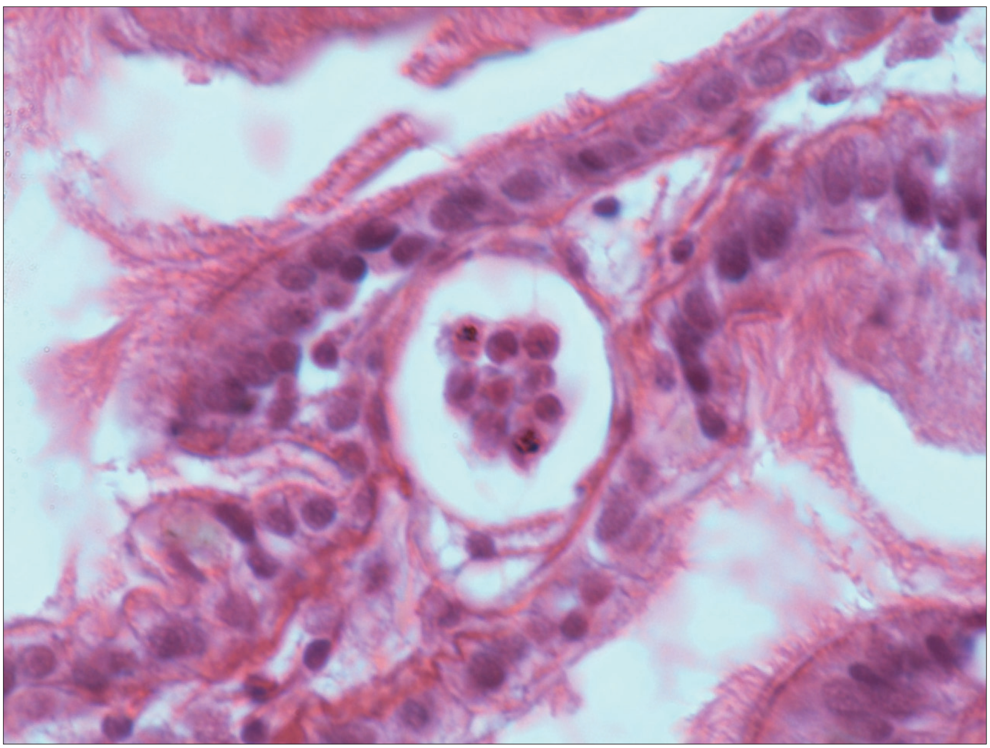


Fig. 29 *Perkinsus* trophozoites in histological preparation

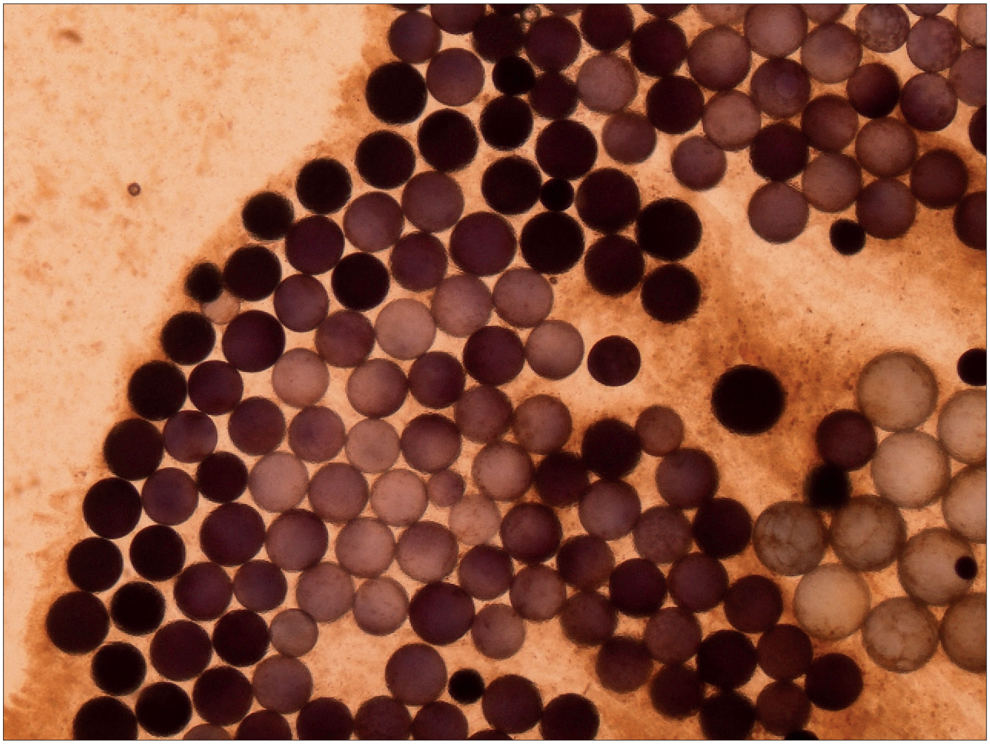


Fig. 30 *Perkinsus* hypnospores cultured in RFTM and stained with Lugol's iodine

Mussels did not show any clinical signs except for the gaping of shells and morbidity. Morbid mussels appeared weak and reacted poorly towards external stimuli, flesh appeared pale, thin and watery and most of the mussels were sexually mature/ spent. The intensity of losses varied from farm to farm and in many farms, strings were virtually empty. On an average, 60-80% loss has been estimated in the farms.

An OIE listed intracellular protozoan parasite, *Perkinsus olseni* was observed in bivalve samples collected from the affected areas. Prevalence of the parasite in the green mussel during the mortality episode (2016) was 100% while it was 39% during 2015. *P. olseni* infections with high prevalence were also observed in the resident clam, *Paphia malabarica* collected during both the years from the area.

In RFTM culture assay, enlarged, blue-black hypnospores were visible under the microscope following staining with Lugol's iodine. Intensity of infection in most of the cases were ascertained as "medium to high" in Mackin's scale.

The histopathology studies revealed mature trophozoites with prominent nuclei with characteristic "signet ring" stages. Fluorescent *in-situ* hybridization distinctly showed the presence of *P. olseni* within the host tissues.

In molecular analysis, specific DNA bands were observed in agarose gels indicative of the presence of *P. olseni*. BLAST analysis of the generated nucleotide sequences made hits with known sequences of *P. olseni* confirming the authenticity of our findings. Further, in the phylogenetic tree (Neighbour Joining) drawn with the sequences of other known *P. olseni*, the present isolates clustered together emphasizing their molecular identity.

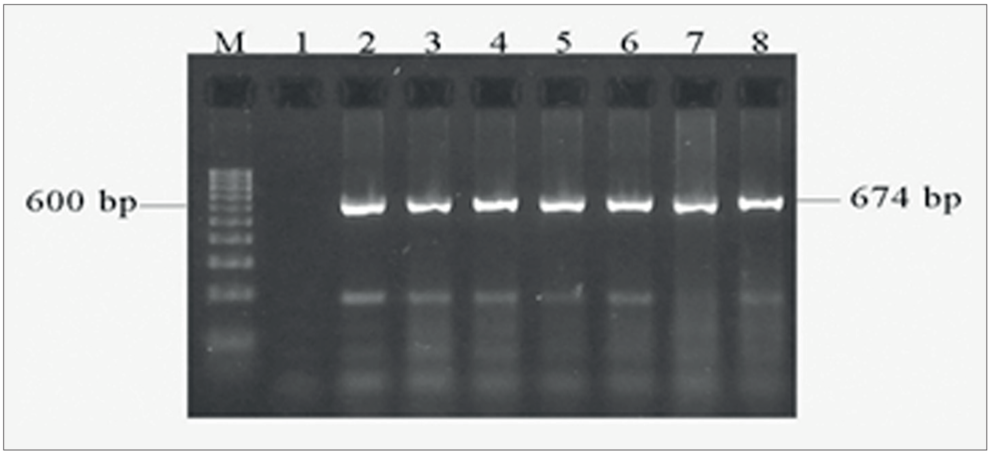


Fig. 31 Gel image of *Perkinsus olseni*

Role of *P. olseni* in the mortalities: *P. olseni* has been known to cause serious mortalities in bivalve populations all over the world. Higher temperature and salinities are always stressful and such conditions are known to increase the pathogenicity/ virulence and associated mortalities in *Perkinsus* infections (Villalba *et al*. 2004, Queiroga *et al*. 2016). Higher temperatures increase metabolic rates, thereby draining energy reserves while higher salinities often induce spawning, an energy demanding process causing physiological stress in animals. The stress caused by elevated temperature and salinities suppresses immune responses thereby triggering and sustaining a pathological assault by the parasite leading to mortality. *Perkinsus* infects and destroys the connective tissues and the mass destruction of cells in vital organ systems eventually leads to mortalities (Anderson 1996). When mussels are weakened due to environmental stress (high temperature and salinity), the susceptibility of mussels to this pathogen increases, resulting in very high mortalities.

Infective stages of the parasite released from dead and decaying mussels act as the source of further infection and proximity of farms greatly enhances the possibility of disease transmission. Since clustering of farms in a limited area and stagnation of water increases the possibility of infection, it is desirable to avoid it.

3.7 Seed sourcing protocols

The mussel farming industry in Kerala collects wild spat from the intertidal and sub-tidal mussel beds during low tide for seeding mussel ropes. Wild mussel spat must be collected without adversely affecting the wild population and the environment. The following advisories are to be followed for handling and transport of mussel seeds in an optimum manner. An infographic of mussel seed collection and transport is shown in Fig.32-33 in English and Malayalam.

Collection of mussel seed

- The mussel seed bags should be soaked in seawater prior to seed collection for at least 6 h and washed thoroughly.
- Mussel seed should be collected during late evening or early morning to avoid direct sunlight
- Desiccated, stunted, unhealthy seed in the intertidal exposed area should be avoided/ discarded
- Seeds of 15-25 mm size are ideal for farming, larger mussels and smaller spat may be avoided
- Good quality seeds can be collected during low tide from intertidal and subtidal mussel beds
- The seeds should be collected with minimum disturbance using a chisel from intertidal and subtidal areas.
- The seeds attached to adult mussels in subtidal collections should be gently detached without damaging the byssus threads.

Handling of mussel seed

- The harvested seed should be kept under shade, kept cool, under wet conditions (by spraying seawater) and out of the sun at all times.
- The seeds should be gently washed in seawater in a net bag of appropriate mesh size to remove adhering sand/ sediment
- The seeds should be gently packed in a wet and cleaned gunny bag, not exceeding 20 kg

- The bags should not be transported in water
- Transportation of seed in wet gunny bags and moistened with cool seawater periodically (2 hourly) for maintaining the humidity and for reducing desiccation from wind during transit.
- Seed bags should be handled gently, avoiding dropping/ throwing while loading and unloading.
- The seed bags should be transported preferably during the cool hours of the night, therefore, the harvesting of seed from natural beds should be scheduled accordingly
- Harvested seed should be transported quickly without holding them under exposed conditions
- Bags should not be stacked one above the other to avoid compression and to avoid possible physical damage
- Seed bags should be transported under covered conditions during evening and night in a vehicle

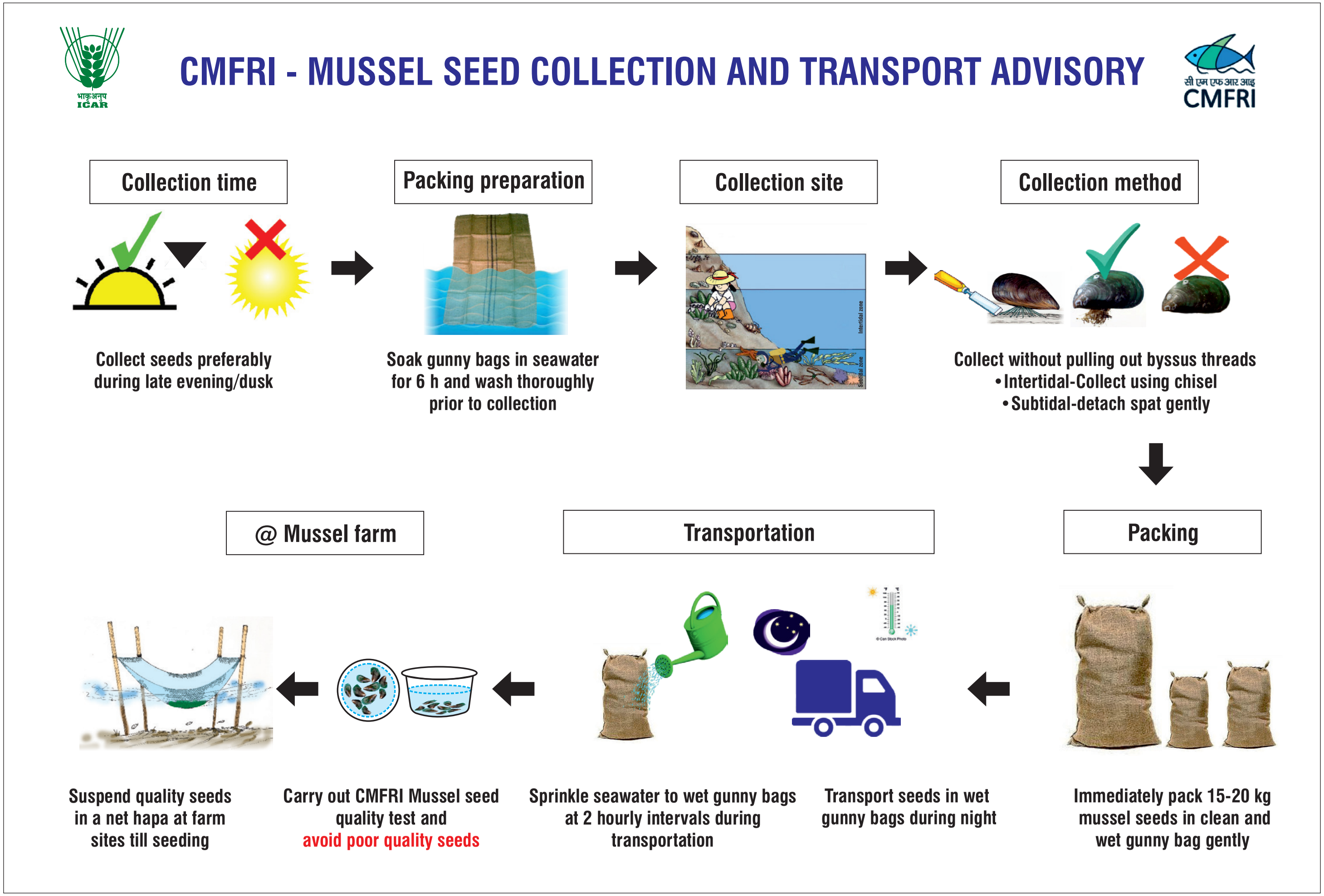


Fig.32. Do's and Don'ts for harvesting seed from natural mussel beds

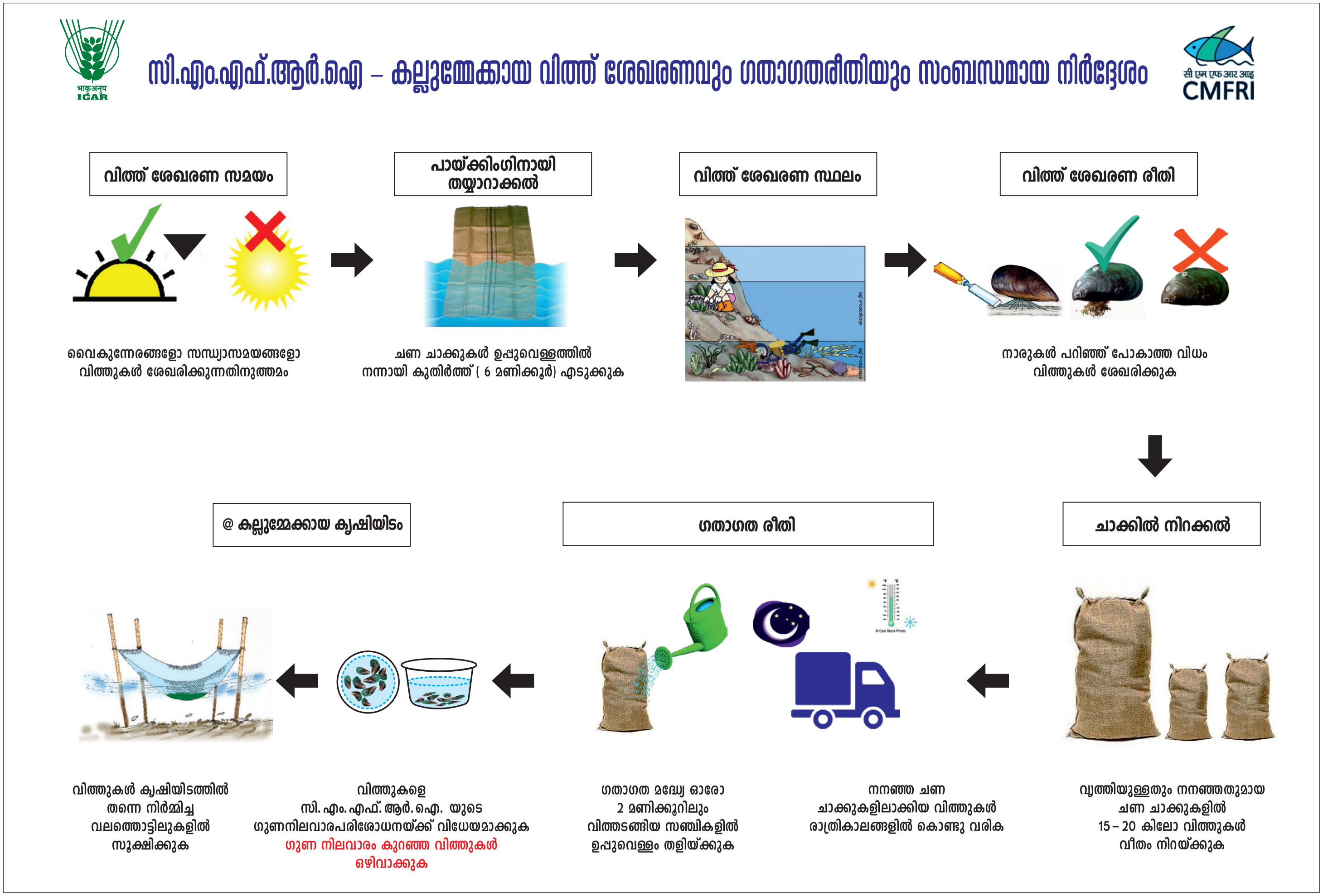


Fig.33. Do's and Don'ts for harvesting seed from natural mussel beds (in Malayalam)

3.8 Seed quality

The mussel seed quality test, based on byssus attachment indicated that, in case of poor-quality seeds, there was a delay in byssus attachment to the substratum, whereas, good quality seeds rapidly attached by byssus production. Fig. 34 provides an infographic of the protocols to be followed for conducting the mussel seed quality test. Fig. 35 provides the mussel seed quality test reference table at the farmer's level for assessing the quality of seed.

Mussel spat quality

- The farmers are advised to carry out the mussel seed quality testing in the farm site
- Record the seed stress percentage as per the protocol in Fig.34.
- Determine the seed quality score by referring to Fig.35.
- Suspend the good quality seeds supplied to the farmer in a net hapa at farm site till seeding
- Record seed count per 100g for arriving at the seeding density
- Record the source of mussel seed for traceability
- Avoid poor quality seeds for farming

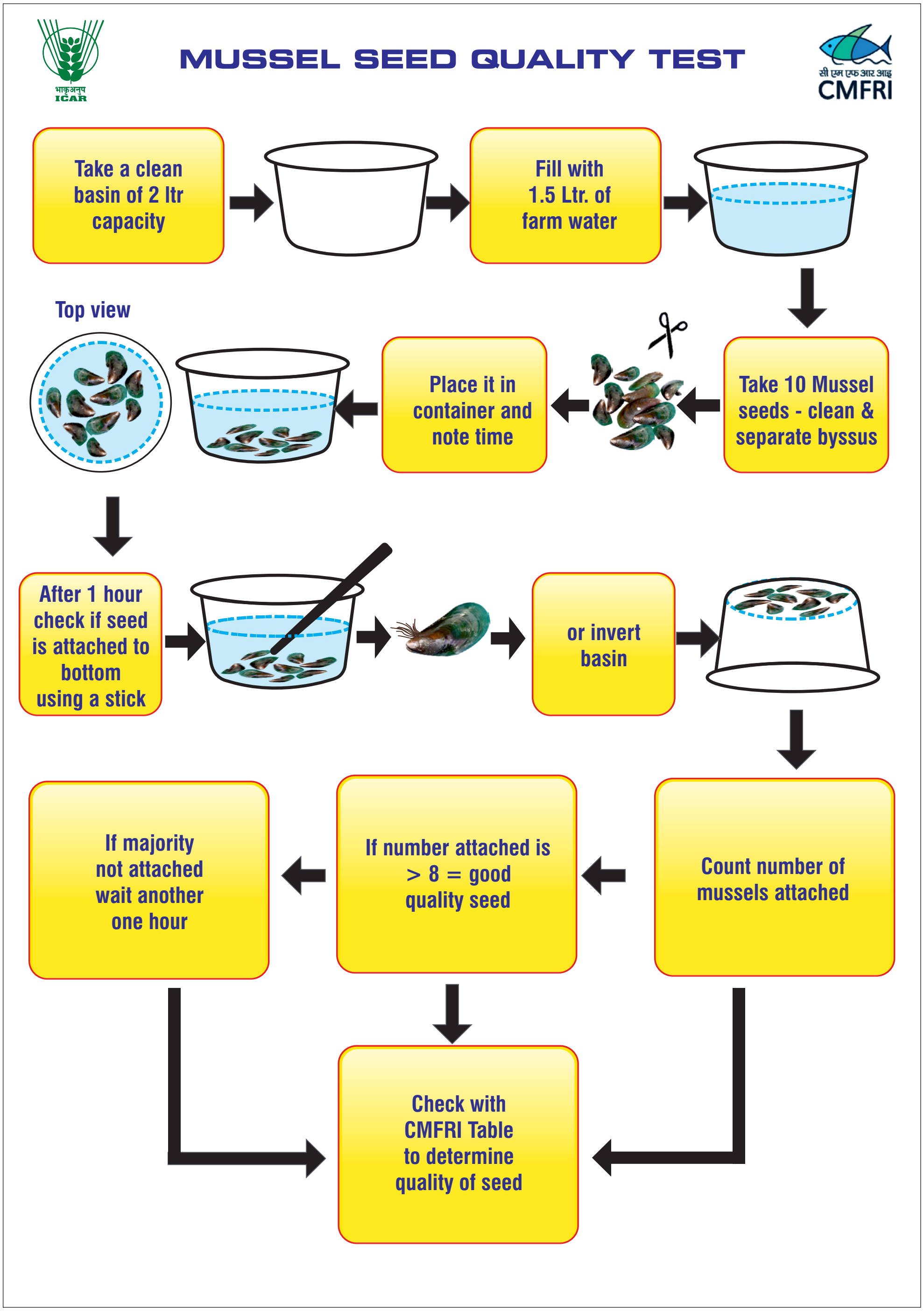


Fig.34. Mussel seed quality test protocol

CMFRI Mussel Seed Quality Test Reference Table

Mussel seed transportation time

Test Hours	1 hr	5 hr	10 hr	20 hr	30 hr	36 hr
	Table 1. Intertidal mussel seed					
	% of seed attachment					
1	100	100	100	100	87	80
2					97	90
3					100	100
	Table 2. Subtidal mussel seed					
1	100	65	63	10		
5	100	90	87	40		
10	100	100	100	40		

GOOD

MODERATELY GOOD

POOR

Fig.35. Mussel seed quality reference table

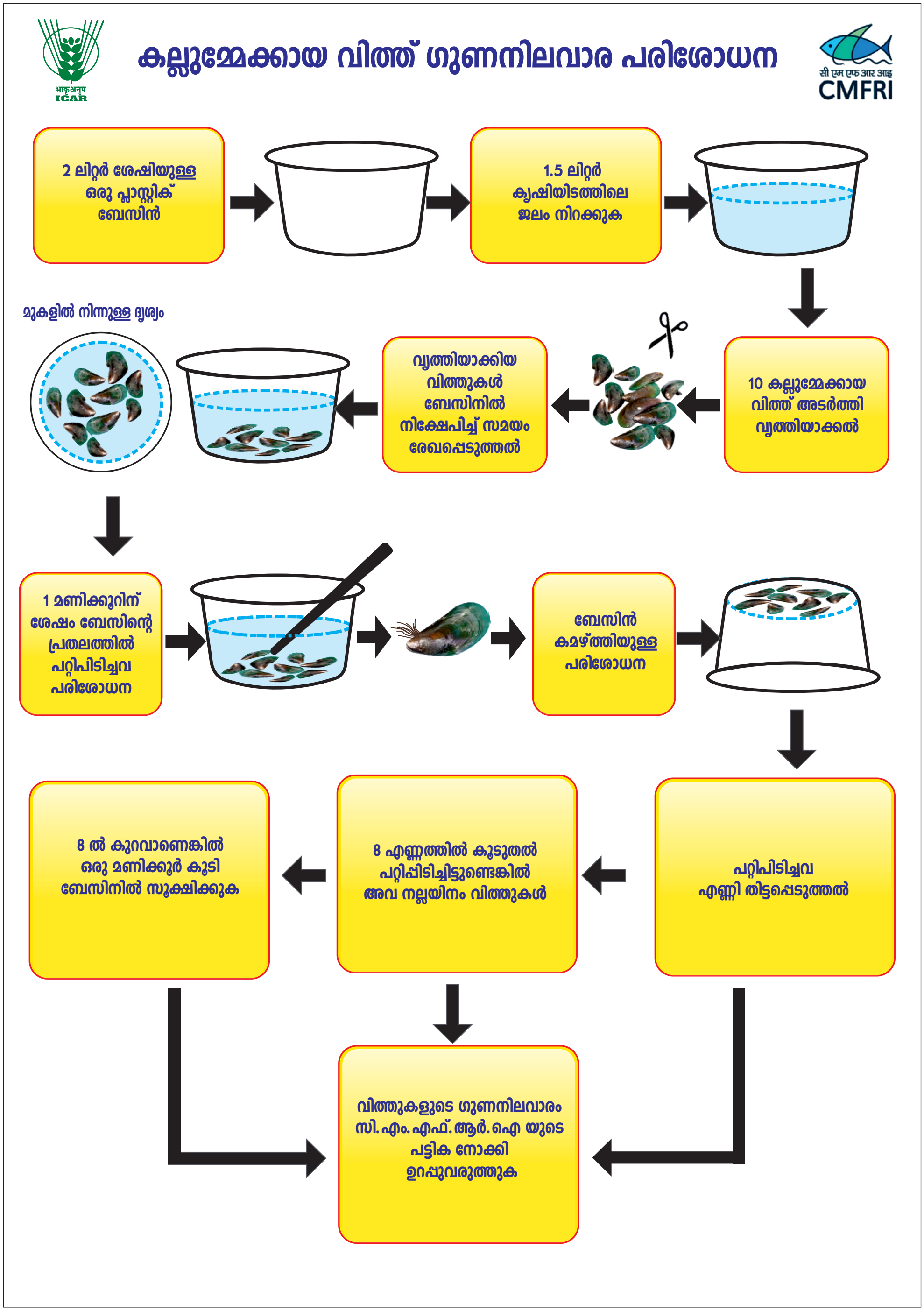
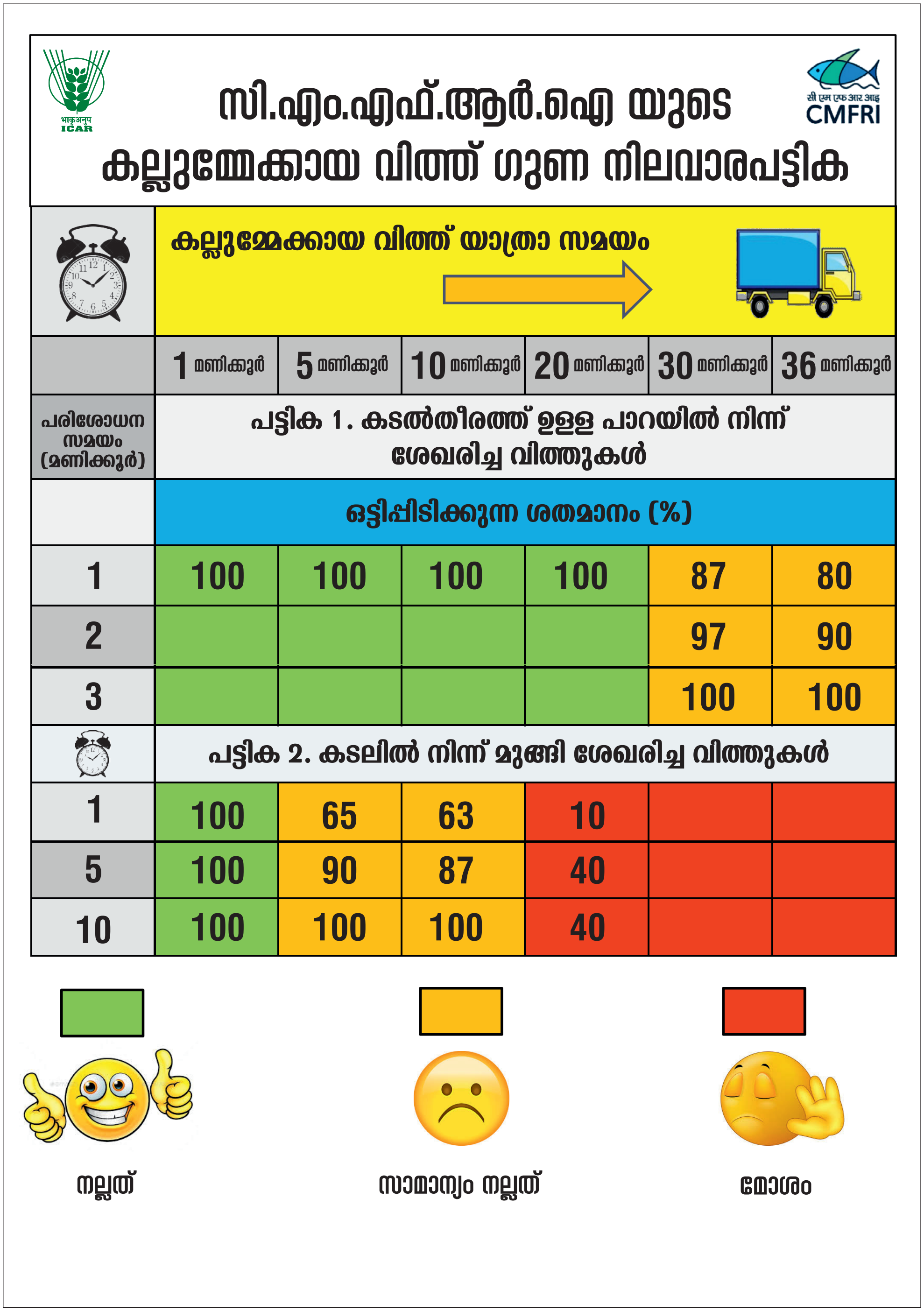


Fig.36. Mussel seed quality test protocol (in Malayalam)



സി.എം.എഫ്.ആർ.ഐ യുടെ കല്ലുമ്മേക്കായ വിത്ത് ഗുണ നിലവാരപട്ടിക

കല്ലുമ്മേക്കായ വിത്ത് യാത്രാ സമയം

പരിശോധന സമയം (മണിക്കൂർ)	1 മണിക്കൂർ	5 മണിക്കൂർ	10 മണിക്കൂർ	20 മണിക്കൂർ	30 മണിക്കൂർ	36 മണിക്കൂർ
	പട്ടിക 1. കടൽതീരത്ത് ഉള്ള പാറയിൽ നിന്ന് ശേഖരിച്ച വിത്തുകൾ					
	ഒട്ടിപ്പിടിക്കുന്ന ശതമാനം (%)					
1	100	100	100	100	87	80
2					97	90
3					100	100
	പട്ടിക 2. കടലിൽ നിന്ന് മുങ്ങി ശേഖരിച്ച വിത്തുകൾ					
1	100	65	63	10		
5	100	90	87	40		
10	100	100	100	40		

നല്ലത് സാമാന്യം നല്ലത് മോശം

Fig.37. Mussel seed quality reference table (in Malayalam)

4. Recommendations for Padanna

1. Farm registration and licensing practices should be followed by the Department of Fisheries, Kerala State, in collaboration with the local Panchayat.

2. Area of each farm unit should be restricted to 5x5 m, with a capacity to support 100 ropes.

3. Every year, after the harvest, the rack constructed should be removed from the area. These structures aid in accumulation of silt due to farming. The monsoon would help in flushing the accumulated silt, provided the farm structures are removed.

4. The distance between the unit farms has to be regulated with a minimum of 25 m distance

5. As far as possible the farms should be located in a staggered manner (zigzag) as shown in Fig.38

6. One farming area should be used only for two farming seasons, and in the

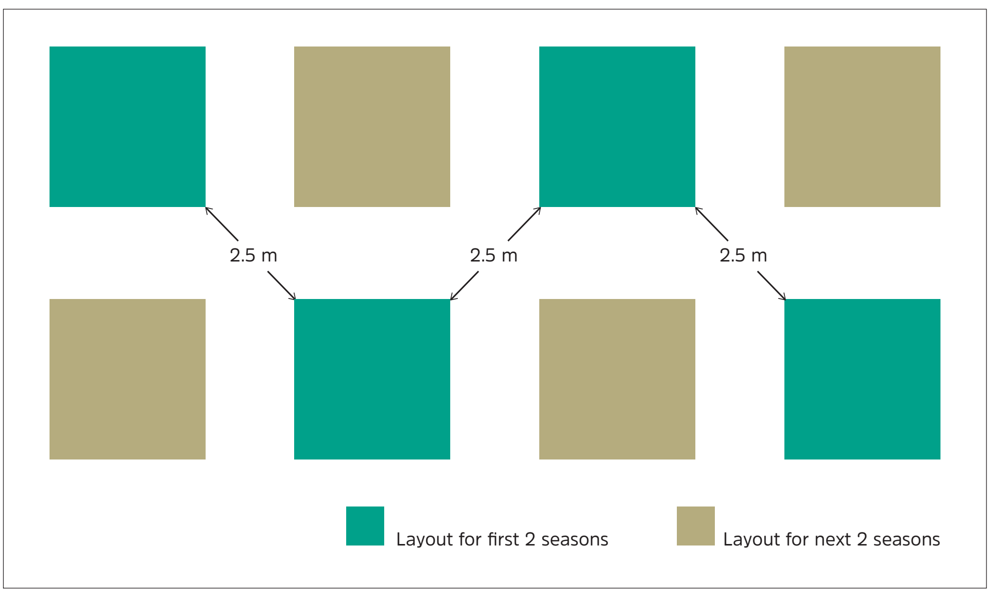


Fig. 38. Recommended layout of the farming units in backwaters

third season the farm unit should be shifted to an adjacent non-farmed area (Fig. 38).

7. The number of farm units has to be reduced by 20 % in Padanna Backwaters as given in Table 5.

Table 5. Proposed no. of units of 25 sq.m. with 20% reduction.

Sl No	Place	No of units in 2015-16 (as per DOF records)	No of units Proposed
1	Cheruvattur	81	65
2	Padanna Kadappuram*	127	102
3	Padanna	476	381
4	Vadakkekkad	128	102
5	Veliyaparambu	20	16
6	Edayillakadu	506	405
7	Thrikkaripur	34	28
8	Madakka	264	211
9	Kannuveedu	126	101
	Total	1762	1410

* To be redistributed into current farming area

8. This recommended reduction in number of farm units by 20% and increase in the distance between the farm units from the present average of 6.3m to 25m, is expected to maintain the productivity at 45±2 tonnes/ ha per unit area.

9. Authorities may consider the creation of a narrow mouth opening to the sea along the western boundary of Padanna backwaters.

10. The earthen bund has to be modified with provision for free exchange of water using wide diameter concrete pipes/ bridge on pillar for proper water flow with respect to: i. Thekkekkad Bund road; ii. Edayilakkadu road iii. Udumanthala Madakka road (Fig.39).

11. Of the three bund roads, the Edayilakkadu road does not have any provisions for water exchange at all. The other two has limited water exchange provisions. Water flow facilitates flushing of waste materials, reduce temperature and address hypersalination.

12. Farmers have to be trained in ascertaining seed quality using seed quality test

Fig. 39 Map showing the location of Bunds in Padanna Backwaters

(refer Fig. 34-37) and the seeds passing this test should be used for farming. This training would be provided by CMFRI.

13. Seeds suppliers should be trained to handle mussel seeds during transportation and supply, as careful handling and transportation are essential to avoid stress (refer Fig. 32-33)

14. Seeds should be transported during cool hours (night)

15. Seeds should be under moist conditions.

16. Seeds should preferably be collected from nearby areas to reduce the duration of transportation.

17. The seed supplier must inform the source of the seeds to the farmer

18. Since there is high demand for seed, the CMFRI is working towards scaling-up the mussel hatchery technology developed earlier to a commercial level. But this would take another year and can at best meet only a small percentage of the demand in the next 5 years. CMFRI is working to develop model hatcheries which can supply seeds to farmers.

19. Recovery / Rebuilding of the current farming practice from the present decline has to be a gradual process.

20. Compensation package for loss of farmed stock due to the current environmental catastrophe by the government should only be given to those units which are adhering to the rules and regulations recommended herein.

21. CMFRI has to conduct new surveys in the ecosystem to determine additional areas suitable for farming.

5. Guidance for Good Mussel Farming Practices

In India, the technology for farming mussels was developed during 1970s and was subsequently popularized at various locations along the country's southeast and southwest coasts by Central Marine Fisheries Research Institute (CMFRI). Green mussel, *Perna viridis*, and brown mussel *Perna indica* are the commercially important species. Green mussels are farmed in marine areas of estuaries and backwaters since 1996 on a commercial scale. The farming method can include suspended cultivation from rack (fixed), raft (floating), longline (floating) or direct spreading onto the estuarine/ seabed. Estuarine areas with good salinity, less turbulence and shallow depths are the most popular farming areas.

A key component in this initiative is to promote a culture of sustainable farming, by improving management practices. Achieving high standards of environmental awareness will assist in developing aquaculture business in an ecologically sustainable manner.

In the backdrop of recent setbacks in the mussel farming sector, the following guidance for good mussel farming practices are proposed for a review of management procedures.

The bivalve aquaculture practices require compliance with appropriate farming-related laws as well as environmental, social and food safety regulations.

5.1 Site suitability for mussel farming

- Mussel farming should be undertaken in farming sites classified (Annexure I) by competent authority/s (Annexure II). Source of pollution to bivalve growing areas should be identified through shoreline sanitary surveys. The farming waters should be tested for coliforms, the indicator for sewage pollution.

- Mussel farming in India typically requires 4-6 months for the spat to grow to a suitable market size. The important environmental considerations for farming include salinity (27-35psu), temperature (26-32^{o}C), clear seawater with good phytoplankton production and moderate water current. The site should be free from sewage, domestic and industrial pollution.

- Changes in salinity will affect the survival of marine mussels in estuarine farming sites during seasonal monsoons. Farms should be sited as possible in areas having optimum salinity range, avoiding areas with high inflow of fresh water.

- Mussel farming relies entirely on natural food sources. These filter feeders are net extractors from the water; the main component of food is phytoplankton and small organic particles in the water. In the process the mussels remove plankton and return some of it to the water as faeces or pseudofaeces.

- The ability of environments to support dense populations of cultured filter feeders varies with the tidal flow, nutrient status, primary production and other factors. Hence the effects of filter feeder farming on the nutrient and phytoplankton status of waters supporting the farms should be determined scientifically.

- The research organizations should appropriately scale the farming operation in a given site with the number of farms, number of culture ropes, flushing rate and primary production so as to maintain the ecosystem's natural function.

- When they are cultivated in high densities, there is potential for the production carrying capacity of the water body to be exceeded.

- Mussel farming when undertaken by suspended farming methods using fixed rack or floating raft or on-bottom farming techniques should be based on scientific advice fulfilling all legal and regulatory requirements (leasing, licensing and registration).

5.2 Regulatory Compliance (Leasing, Licensing, Registration)

- Mussel farming sites in estuarine and coastal waters should be considered based on the site selection criteria set for *Perna viridis* since they are efficient filter feeders. The research organizations should review regularly the location of farming, area and the number of farms scientifically with respect to the carrying capacity of the water body.

5.2.1 Leasing

- The farmer/self-help group should ensure that the farming operation is according to all legal and regulatory requirements.

- Aquaculture lease should be sought from the Village Panchayat/ local self-government institutions, having leasing rights over the water body/ farming site. Leasing rights should specify the area of farm, the farm size, the duration of farming, the type of farming and other lease terms.

- A lease amount should be paid as rent for the duration of farming. In some cases, licensees holding an aquaculture lease, should deposit a bond with the local self-government institutions which will cover site clean-up costs in the event that the site is abandoned.

- The local self-government should consider providing access to the extent that is practicable for other fishing or navigational activities while leasing sites for bivalve farming for minimizing conflicts.

5.2.2 Licensing

- Operating licenses for mussel aquaculture should be assessed, reviewed and issued by the Local Self Government (Panchayat) of the respective state based on the advice of the Research Institute.

- Licenses are subject to annual fee and conditions set by Department of Fisheries (DoF).

- Licensees are required in aquaculture activities to monitor the farming (actual duration, seed source, harvest details, survival) and environmental management measures (removal of farm structures after farming from the site, disposal of farm materials and others). They should ensure that the allocated area should be utilized for mussel farming avoiding conflicts of space allocation with other stakeholders

5.2.3 Registration

Once the leasing rights are obtained the farm should be registered with the Department of Fisheries (DoF).

5.3 Farm construction

- Individuals/ groups (licensees) participating in farming shall maintain all necessary documents for siting, constructing and operating their facilities before farm construction.

- The recommended distance between the farming structure should be maintained for good water exchange

- Material used for farm construction should be inert and non-polluting

- Farmers should cooperate with local communities and cooperate with other land and water users to minimize conflicts

5.4 Farming operation and management

- The farmers should adopt responsible practice in setting seeding densities based on the initial seed size in mussel farming. High stocking densities can be detrimental on a local scale, which has implication for growth rates and yield as well as local food availability.

- The environmental impact of mussel farming includes potential sediment accumulation under the farming structure. Physical presence of the aquaculture facility can change the hydrodynamic conditions and result in a change in sediment characteristics in the immediate vicinity of the facility. Additionally, the accumulation of organic matter has potential implications for benthic biodiversity due to related effects, including oxygen depletion and increased levels of hydrogen sulphide. Therefore, the farm should be maintained and operated to facilitate good circulation and tidal flow. This will disperse or remove the built-up of sediment and pseudofeaces under the structure.

- Biological sampling and benthic environment monitoring should be undertaken by research organizations for minimizing adverse impacts.

- An environmentally friendly approach shall be taken to dispose of waste materials used in mussel farming including polypropylene ropes, poles, nets, trays, concrete dead weights, sinkers etc.

- Growth and survival of the farm stock should be regularly monitored.

- The movement of mussel seed or adults brings with it the risk of introducing infectious diseases and parasites. Monitoring must be undertaken for disease outbreaks so that any spread can be contained.

- Signs of disease or unexplained high mortality levels should be reported to the local self-government as and when observed.

- The local self-government should have a recovery and disposal plan in place for dead mussels in the event of mass mortalities, with identified local services which can be called on to quickly provide assistance

- Algal bloom in the farming site should be reported to the Local self-government

- When algal blooms are observed harvesting should be avoided

- The racks should be dismantled immediately after harvest by removing the horizontal and vertical poles from the farming sites. This helps in flushing the accumulated silt during the monsoon season.

5.5 Post-harvest

- Mussels can be exposed to a wide range of potential contaminants depending on the farming site. During filter feeding these contaminants can accumulate in their flesh, causing them to become naturally contaminated.

- Good cultivation practices therefore require a significant awareness of external threats, in addition to the implementation of responsive internal management applying the most appropriate means of purifying contaminated shellfish (such as relaying and depuration based on the classification of growing waters)

- The depuration process (Chinnadurai *et al*., 2014) normally involves placing trays of shellfish into a purpose-made tank which is then filled with clean seawater or treated seawater to ensure cleanliness. The water is then recycled or operated on a single pass flow-through basis

- For depuration to be effective in removing microbiological contamination, the design of the system and the operation of the entire process must allow mussel to:

    - rapidly resume normal filter-feeding activity and to maintain this for the duration of the process. This requires optimization of physiological conditions;

    - facilitate removal and separation of faecal contaminants excreted by mussel. This requires appropriate design and operation of systems; and

    - avoid any contamination or re-contamination of the mussel during the process. This requires an appropriate quality of seawater used in the process and proper operation of the system.

- Any batch of mussel undergoing purification must be of the same class of production area.

- Relaying involves the transfer of harvested animals to cleaner estuaries or inlets for self-purification in the natural environment. Mussel can only be held for relatively short periods in depuration tanks but can obviously be maintained for much longer periods in the natural environment. This makes relaying also suitable for treating more heavily polluted shellfish where longer periods (two months) are required to remove heavy contaminant loads.

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Annexure I

EU classification criteria for classification of shellfish harvesting area

CLASS[1]	MICROBIOLOGICAL STANDARD[2]	POST-HARVEST TREATMENT REQUIRED
A	Samples of live bivalve molluscs from these areas must not exceed, in 80% of samples collected during the review period, 230 *E. coli* per 100 g of flesh and intravalvular liquid. The remaining 20% of samples must not exceed 700 *E. coli* per 100 g of flesh and intravalvular liquid[3]	None
B	Live bivalve molluscs from these areas must not exceed, in 90% of the samples, 4600 MPN *E. coli* per 100 g of flesh and intravalvular liquid. In the remaining 10% of samples, live bivalve molluscs must not exceed 46 000 MPN *E. coli* per 100 g of flesh and intravalvular liquid[4]	Purification, relaying or heat treatment by an approved method
C	Live bivalve molluscs from these areas must not exceed 46 000 *E. coli* MPN per 100 g of flesh and intravalvular liquid[5]	Relaying or heat treatment by an approved method

Source: FAO and WHO (2018)

1. The competent authority (= responsible authority) has the power to prohibit any production and harvesting of bivalve molluscs in areas considered unsuitable for health reasons. Harvesting may not be undertaken from areas not meeting the requirements for Class A, B or C.

2. The reference method is given as ISO 16649-3.

3. Regulation (EC) No 854/2004 as amended by Regulation (EU) 2015/2285.

4. Regulation (EC) No 854/2004 as amended by Regulation (EC) No 1021/2008.

5. From Regulation (EC) No 854/2004.

Classification Criteria Under The U.S. NSSP For Shellfish Harvesting Area

CLASSIFICATION	FAECAL COLIFORMS PER 100 ml WATER		TREATMENT REQUIRED
	GEOMETRIC MEAN[1]	90% COMPLIANCE[2]	
Approved Areas[3]	≤ 14	≤ 43	None
Restricted Areas[4]	≤ 88	≤ 260	Depuration[5] or relaying in an approved area
Prohibited Areas	No sanitary survey, or conditions not met for approved or restricted areas[6]		Harvesting not permitted

Source: FAO and WHO (2018)

1. Or median;

2. Values for 5-tube decimal dilution test – a different 90 percent compliance is given for the 3-tube MPN and mTEC membrane filtration tests;

3. Determination of approved area status must be based on a minimum of 15 samples from each monitoring station.

4. Conditionally restricted areas may be declared where these are subject to predictable contamination events: such areas are closed for harvesting during contamination events and for a period afterwards to permit natural cleansing.

5. Depuration and purification are alternative terms applied to the process by which bivalve molluscs are held in tanks of clean seawater under conditions that maximize natural filtering activity, and which results in expulsion of intestinal contents, enhancing separation of the expelled contaminants from the bivalves, and preventing their recontamination (FAO, 2008).

6. Considerations other than the concentration of contaminants may be used to declare an area prohibited.

Annexure II

Long-term monitoring strategy for bivalve growing waters

- Identification of a competent authority/s for monitoring, evaluation, classification of bivalve waters and for product certification
- Constitution of a panel by the identified competent authority comprising of officials from EIC/EIA, FSSAI, MPEDA, Department of Fisheries, ICAR-CIFT and ICAR-CMFRI for periodic review

Activity	Competent authority & role
Shellfish safety monitoring and certification programme by accredited and competent authority/s for consumer safety	Bivalve growing areas certification Bivalve Product Quality assurance certification by EIC/EIA
Competent authority/s should appropriately classify the bivalve areas	Monitoring, Evaluation and Classification (as approved, restricted and prohibited) of bivalve growing areas may be carried out broadly based on: Growing water test results or Bivalve tissue test results or a combination of both by EIC/EIA/ MPEDA on the advice of CMFRI Shoreline surveys and collection and testing of bivalve tissue and water samples for monitoring and evaluation by EIC/EIA/ CMFRI in partnership with NABL accredited laboratories
Recommend closure of bivalve fishery/ farming when contamination is detected	Bivalve growing areas closure by EIC/EIA+DOF Where the quality criteria are not met, appropriate actions should be taken as deemed by the competent authority. In following up, consideration should be given to detention, recall and further processing in a manner to eliminate the hazard from implicated bivalve lots. In addition, assessment of the status of growing areas and/or establishment controls should be undertaken.
Classification of new/ prospective bivalve farming site	If a prospective farming site is not already part of a certified shellfish-growing area, Competent authority/s must conduct a shoreline survey to classify the area. CMFRI/ EIC/EIA.
Depuration of bivalve	When areas are not fully approved, relaying and depuration may be followed as appropriate, subjected to suitable controls. EIC/EIA/ MPEDA+DOF.

- Traceability of bivalve products

Activity	Competent authority & role
Tracing bivalve shellfish through the supply chain to consumers, ensuring food safety	EIC/EIA/FSSAI/MPEDA, in partnership with Local regulatory body with respect to food safety may be entrusted with enforcement
Possible to locate products to inform recalls in case of a health concern	Identified competent authority/s –EIC/EIA, FSSAI

- Research and outreach activities

Activity	Competent authority & role
Model for monitoring bivalve/ shellfish growing water bodies and scientific advisories Carrying capacity, advisories on number of bivalve farms	ICAR-CMFRI ICAR-CMFRI
Bivalve health	ICAR-CMFRI
Stock assessment and catch quota for bivalve fishery management leading to sustainability certification	ICAR-CMFRI & MPEDA
Environmental interactions (HAB, oil spill, pollution)	ICAR-CMFRI & CIFT Disseminate information on the pollution/ PSP/ Red tide status of bivalve beds for facilitating timely closures.

Guidance for Good Mussel Farming Practices in India based on a case study from Kerala

Mussel farming technology which was developed in India by CMFRI saw a rapid uptake by women self help groups in Padanna Backwaters of northern Kerala. This policy document investigates the reasons for the recent rapid decline in farmed green mussel production in the region. The adoption of sustainable aquaculture practices in Padanna Backwaters by improving the quality of seeds, enhancing the flushing rates, modifying the farm layout and reducing the farming density per unit area are among the 21 recommendations proposed by CMFRI to tackle these challenges. These recommendations are complementary to the global guidelines for Best Aquaculture Practices for mussels.

भाकृअनुप
ICAR

सी एम एफ आर आइ
CMFRI

Indian Council of Agricultural Research
Central Marine Fisheries Research Institute

Post Box No.1603, Ernakulam North P.O., Kochi-682 018, Kerala, India.
Phone: +91 484 2394357, 2394867
Fax: +91 484 2394909
E-mail: contact@cmfri.org.in

www.cmfri.org.in

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